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BULLETIN OF
THE NEW YORK ACADEMY
OF MEDICINE

OCTOBER 1939

PROTEINS AS CHEMICAL SUBSTANCES AND AS BIOLOGICAL COMPONENTS

EDWIN J. COHN

Harvey Lecture, January 19, 1939

THE importance of the diverse class of molecules that we know as proteins has in recent years become ever more apparent. Proteins were regarded as chemical substances even a century ago. Thus in 1838, Mulder reported elementary analyses of various proteins, including egg and serum albumins, and estimated minimal molecular weights of over 50,000 on the basis of their sulfur and phosphorus content.[1]

The sulfur and phosphorus analyses of Mulder were in error, and so were the atomic weights that he employed. But the concept of proteins as chemical substances is implicit in his calculations.

Three years later† than the number containing Mulder's analyses there appeared a discussion in Liebig's Annalen of a communication from the French scientist Denis to Liebig in which is described the separation of blood proteins into those soluble in water, and those which were only soluble in salt solutions, that is, into albumins and globulins.[2] A century ago, therefore, there was a beginning not only of the characterization of proteins as chemical substances, but the fractionation of tissues into their

† In the same volume is a communication of Dr. Bence-Jones[3] on the nitrogen containing foodstuffs of the plant world, on the albumins of the brain, and of the egg yolk, reporting analyses carried out in part with Liebig in the Chemical Laboratory in Giessen.

TABLE I*

	Fibrin	Albumin v. Eiern	Albumin v. Serum
Kohlenstoff .	54,56 —	54,48 —	54,84
Wasserstoff .	6,90 —	7,01 —	7,09
Stickstoff .	15,72 —	15,70 —	15,83
Sauerstoff .	22,13 —	22,00 —	21,23
Phosphor .	0,33 —	0,43 —	0,33
Schwefel .	0,36 —	0,38 —	0,68

Auf die kleinere Zahl der Atome von Schwefel und Phosphor berechnet, erhält man:

	Fibrin u. Albumin v. Eiern		Albumin v. Serum	
	Atome	in 100 Th.	Atome	in 100 Th.
Kohlenstoff . . .	400 —	54,90 —	400 —	54,70
Wasserstoff . . .	620 —	6,95 —	620 —	6,92
Stickstoff	100 —	15,89 —	100 —	15,84
Sauerstoff	120 —	21,55 —	120 —	21,47
Phosphor	1 —	0,35 —	1 —	0,35
Schwefel	1 —	0,36 —	2 —	0,72
Atomgewicht	= 55692,61	—	= 55893,78.	

protein components.

In the intervening century more and more proteins have been isolated from biological systems, purified and characterized by ever improving methods. The reserve proteins of the seeds of plants were presumably the first to be observed in a crystalline state. Until recently the proteins most investigated were the seed globulins, the proteins of the body fluids, such as hemoglobin, the protein of the blood corpuscle which carries oxygen to the tissues, the albumins of serum and of egg white, the casein of milk, the fibrinogen of plasma responsible for the clotting of the blood, the serum globulins, bearers of immunity, and the globulin of muscle, myosin, concerned with muscular contraction. The latter protein has been repeatedly investigated from many points of view and may be said to represent the transition to the study of tissue proteins; that is, to those molecules to which we now look as elements of morphological structure in the body.

That certain hormones and enzymes are protein in nature has only recently become apparent. The iodine-containing hormone of the thy-

* Reproduced from Mulder.[3]

roid gland, the active principle of which is thyroxin or some closely related configuration, is thyroglobulin. In thyroglobulin as in hemoglobin, the active configuration is a prosthetic group containing an element—iodine in the one, iron in the other—not found in most proteins.

Another hormone which has been proven to be protein in nature is the insulin effective in the treatment of diabetes, discovered by Banting and crystallized by Abel. The large number of studies in different laboratories on insulin have thus far failed to reveal, as du Vigneaud has pointed out,[4] any prosthetic group and thus demonstrated that a pure protein may serve as a hormone.

It is over a decade since Sumner[5] first crystallized an enzyme, the urea splitting urease, and found it to be a protein. Northrop and his colleagues' crystallization of pepsin, pepsinogen, trypsin, trypsinogen, and chymotrypsin,[6] all digestive enzymes, and Sumner's of catalase[5] followed, and it has become apparent from much of Northrop's[6] and of Bergmann's work[7,8] that the specificity of enzymes depends upon the chemical structure of the protein molecule.

The vitamins were discovered as a consequence of investigations of the nutritional requirements of the body. Therapy consisted in adding vitamins to the diet, but their physiological function and morphological locus remained unknown. Recently it has been demonstrated that riboflavin which is part of the vitamin B_2 complex is the prosthetic group[9] of the yellow enzyme of Warburg[10] and that the carotenoids, which are known as vitamin A_1 and A_2 are the prosthetic group[11] of visual purple, a protein with a molecular weight of about 250,000.[12]

The bearers of immunity have long been associated with the globulin fractions of the serum. In how far the very great specificity of immune reactions inheres in these, in how far in the specific polysaccharides[13] with which antigens are associated, is still being investigated.

One must now add to the proteins of importance in physiology, pharmacology, morphology and pathology, at least certain of the viruses hitherto the concern of bacteriology. The virus sizes were known as the result of a series of ultrafiltration studies by Zinsser,[14] Bechhold,[15] Elford,[16] and others,[17] but it remained for Stanley[18,19] to isolate the virus of tobacco mosaic disease, and demonstrate that it was protein in nature. The huge size of tobacco mosaic virus has been deduced from ultracentrifugal studies[20] and its long asymmetrical shape has been investigated by the method of double refraction of flow.[21,22] Like the myosin

of skeletal muscle,[23] tobacco mosaic virus appears to have a length greater than 5,000 Angstroms, a value which may be contrasted with a diameter of 1.5 Angstroms of the carbon atom, and 44 Angstroms for proteins of the molecular weights of pepsin, insulin and lactoglobulin provided they be considered spherical. Not all of the proteins of body fluids are, however, to be regarded as spherical, and not all viruses as elongated. Among the former fibrinogen, the protein involved in the clotting of blood, is elongated and among the latter the newly crystallized Bushy Stunt virus of the tomato[24] is nearly spherical.[25]

The diversity of the functions subserved by protein molecules is not greater than the diversity in their sizes, shapes, and stereochemical configurations. Obviously some of the methods that must be employed for the study of these protein molecules were not envisioned by earlier chemical and biochemical investigators. New methods have been developed as the new molecules were discovered and the numbers of them that have been and are being perfected is increasingly gratifying.

The Polar and Non-polar Groups of Proteins

Despite the diversity in the form and function of proteins, the number of well recognized chemical configurations which constitute the side chains of the amino acids and therefore of the proteins is by no means large. Without considering here either the methods that are available for the estimation of the amino acid composition of proteins, or the manner in which the peptide chain is ordered in the protein, one may consider the nature of the various configurations which constitute the reactive groups of proteins. In Table II molecular weights are given for a few proteins whose amino acid compositions have been partially apprehended. Only the analytical procedures for sulfur and sulfide sulfur, for cystine and methionine, and for tryptophane and tyrosine have been employed in estimating their minimal molecular weights which have elsewhere been compared[26,27] with those deduced from the osmotic pressure measurements of Sörensen, Adair and Burk, and the beautiful ultracentrifugal studies of Svedberg and his coworkers.[28] The number of sulfhydryl and methionyl, of indole and phenolic hydroxyl groups per mole were then computed. The estimates of imidazol groups derived from histidine analysis and of guanidine groups derived from arginine analysis may be considered fairly accurate. But the estimates of $-NH_3^+$, equivalent to ε-amino groups, may well be low since they are based upon

determinations of lysine, often estimated by difference, and besides do not include the hydroxylysine whose isolation from protein has recently been reported.[29]

The guanidine group is the most basic of which proteins are possessed, and the properties of the protamines must in large part be considered to depend upon this configuration. The ε-amino group dissociates with a strength which varies widely with its position in the molecule, but is in any case also strongly basic, whereas the imidazole group present in such large amounts in hemoglobin dissociates at more nearly neutral reactions. These three configurations alone bear positive charges in acid solutions, are responsible for the cationic properties of the proteins, and are present in such amounts as quantitatively to account for their acid combining capacities.[30,31]

The dicarboxylic acids yield free carboxyl groups when bound in peptide linkage. However, a portion of the dicarboxylic acids, equivalent to that revealed by determinations of ammonia, are considered to be present in the native protein as amides of the type of asparagine and glutamine. The estimate of carboxyl groups, the only acid groups (if we omit from consideration those of phosphoric acid) which dissociate in relatively acid solution and bear negative charges at neutral reactions must be considered less satisfactory, not only because of the analytical procedures that have thus far been employed, but because the result is a difference between various analytical procedures.

Although only carboxyl groups presumably bear negative charges at acid and neutral reactions, the phenolic hydroxyl group of tyrosine and the sulfhydryl group of cysteine yield negatively charged groups and may therefore be considered to add to the anionic valence of proteins, in so far as these configurations exist[31,32,33,34,35] in the native molecule. In the case of many proteins there is no evidence of free SH groups in the native state, but in certain others, such as myosin,[23,33] sulfhydryl groups appear to be free, and these should dissociate at roughly the same alkalinities as free phenolic hydroxyl groups.

The simplest of the amino acids, glycine, contributes neither anionic nor cationic properties to the protein in which it is held in peptide linkage. Nor does it contribute a side chain terminating in a polar or nonpolar group. Absence of side chains permits glycine peptides to pack closely in the crystal lattice and presumably is related to their low solubilities. Residues of glycine situated between those of other amino acids

cannot pack equally closely, but rather may be thought of as creating cavities on the surface of the molecule.

Of the side chains which do not lead to ionic configurations the amide groups combined with dicarboxylic acids, the hydroxyl groups of serine, threonine, hydroxyproline, and presumably other hydroxy amino acids, must be considered very polar.* On the other hand, the benzene ring of phenylalanine, and those parts of the pyrrolidine ring of proline that extend from the peptide chain must be considered non-polar.

Aside from its polar nature, the hydroxyl group has significance for the chemistry of the proteins because of its close relation to the solvent water, because of its capacity to enter into ester linkage with acids, being the point of attachment of phosphorus at least in certain phosphoproteins, and because of the possible role, not considered here, of the hydroxyl bond for internal protein structure.

Whereas the hydroxyl groups of aliphatic amino acids and of hydroxyproline, the sulfur of methionine and the nitrogen of the indole group must be considered polar, the configurations of which they are a part reflect also their non-polar groups. Thus tryptophane and methionine behave in many respects like leucine.

The number of CH_2 groups vary in the aliphatic series from glycine to leucine and this will affect interactions with polar and non-polar solvent and solute molecules in various ways.[36,37] Moreover, the spatial arrangements of amino acid residues of various side chain lengths† must have a determining influence on the distance of closest approach of protein molecules both with ions, dipolar ions, and uncharged molecules.

It is of interest to note the differences in the free groups of different proteins (Table II) and to point out the importance that must be attached to the improvement of analytical procedures upon which our notions of the fine structure of the proteins must ultimately rest. Many

* As judged by the solubility ratio in ethanol and water, log N_A/N_o (see Table III), asparagine, glutamine, as well as aspartic and glutamic acids, and all the hydroxy acids studied thus far are nearly as polar as glycine, and all more polar than alanine. Thus the values of log N_A/N_o for glycine, asparagine, glutamine, aspartic and glutamic acids, serine, threonine and hydroxyproline are —3.391, —3.402, —3.466, —2.999, —2.992, —3.362, —3.070, —2.893.

† . . . "the peptides of glycine may be considered as simple cylinders of alternating segments of radii 2.61Å. in the neighborhood of the CH_2 group, and 2:12Å. in the neighborhood of the amide group. The average radius on this basis would be 2.32 Å., which is one-half the distance of nearest approach of parallel polypeptide chains."
"Substitution of an alanyl for a glycyl residue in the peptide chain may be considered as adding a branch extending 1.26 Å. from the cylinder. Valine would extend the branch to 2.52 Å., norvaline or leucine to 3.78 Å., and norleucine to 5.04 Å."[38] Astbury gives "a 'side-spacing' of the order of 4½ to 5 Å.: in most of the x-ray photographs of proteins"[39] (p. 199).
"In terms of the dimension of the CH_2 and CONH groups the packing of peptide chains in the two planes should be given by 4.64 (i.e., 2 × 2.32) and 9.68 (i.e., 2 × 4.84) Å., the amino acids of the protein having hydrocarbon chains, on the average, of the length of valine. The presence of amino acids with longer hydrocarbon chains would, of course, still further increase the distance in this plane between parallel polypeptide chains"[38] (p. 95).

TABLE II

NATURE AND NUMBER OF POLAR AND NON-POLAR SIDE CHAIN GROUPS OF CERTAIN PROTEINS

SUBSTANCE	MOLECULAR WEIGHT	CATIONIC GROUPS			ANIONIC GROUPS			NON-IONIC POLAR GROUPS				NON-POLAR GROUPS			
		Guanidine	Ammonium	Imidazole	Carboxyl	Phenolic-hydroxyl	Sulfhydryl or ½ (S-S)	Amide	Hydroxyl	Methionyl	Indole	Paraffin	Benzene	Pyrrolidine	Glycine
		Number of Groups													
Glycine	75		1		1										
Cystine	240		2		2		2								
Salmin	5,600	*24*			1				4			2		6	
Egg Albumin	35,000	12	12	4	28	8	6	27		12	2	45	12	12	
Insulin	35,000	6	6	18	36	24	*36*	36				81	+	+	
Zein	39,000	4	0	2	10	12	3	*84*	6	6		122	18	*30*	
Gliadin	42,000	9	2	9	18	8	8	*128*	1	6	2	43	6	*48*	0
Hemoglobin	66,700	16	38	*33*		12	4		8	4	4	180	18	12	
Serum albumin	73,000	20	*66*	16	*	18	*36*	56	4		2	134	14	6	
Casein	96,000	21	*42*	16	*146*	†	3	90	6	21	8	156	24	72	6
Edestin	309,000	288	72	42	228	78	36	414	58	48	24	616	60	112	

* The values for the sum of the dicarboxylic acids thus far available are even smaller than demanded by the ammonia analysis. The high base combining capacity of serum albumin suggests the desirability of new studies of the dicarboxylic acids of the serum albumins.
† Too discrepant results to be considered.

In constructing this table the following assumptions have been made relating the groups to the amino acids found on hydrolysis:

Guanidine = Arginine.

Ammonium = Lysine.

Imidazole = Histidine.

Carboxyl = Aspartic + glutamic + β-hydroxyglutamic acid — ammonia.

Phenolic-hydroxyl = Tyrosine.

Sulfhydryl or ½ (S-S) = 2 × Cystine, or alkali-labile sulfur if in excess of cysteine sulfur.

Amide = Ammonia.

Hydroxyl = Serine + threonine + hydroxyproline. The numbers of the reported residues of hydroxyglutamic acid are 3 for egg albumin, 6 for zein, 20 for gliadin and 64 for casein, and not included here, but have been counted as carboxyl groups

Methionyl = Methionine, or total sulfur — alkali-labile sulfur.

Indole = Tryptophane.

Paraffin = Alanine + valine + leucine + norleucine. Threonine and methionine are not included since certain of their non-polar groups are situated between polar groups. The total number of CH_2 groups in paraffin side chains (alanine + serine) + 2 threonine + 3 (valine + methionine) + 4 (leucine + norleucine) is for present estimates on these proteins 181, 324, 378, 152, 648, 474, 576, 2246 respectively for egg albumin, insulin, zein, gliadin, hemoglobin, serum albumin, casein and edestin.

Benzene = Phenylalanine. In tyrosine the benzene rings are situated between polar groups. The number of such benzene rings is given by the number of phenolic hydroxyl groups.

Pyrrolidine = Proline. In hydroxyproline the non-polar groups are situated between the hydroxyl groups and the peptide chain. The numbers of these, included in the number of hydroxyl groups, are 2 for zein, 4 for hemoglobin, 2 for casein and 48 for edestin. The hydroxyl minus the hydroxyproline groups yield, of course, serine plus threonine.

proteins appear to have molecular weights ranging from 34,000 to 42,000. The analyses on the basis of which their percentile compositions have been determined have been,[8,26,40] and will be, discussed elsewhere. Although it must be remembered that in many cases the number of groups of any kind may at this time only be estimates, since the analytical evidence regarding many of these residues is by no means satisfactory, there is none the less an advantage in considering the differences in protein molecules which emerge from the analyses at present available.

Zein and gliadin among those of roughly the same molecular weight are classified as prolamines. That is to say, they are insoluble in water, but soluble in alcohol-water mixtures. Their compositions are known to over 90 per cent, and so it would appear to be significant that they contain but very few basic amino acids and very few free carboxyl groups, but consist predominantly of glutamine and asparagine, of proline and of mono-amino monocarboxylic acids with paraffin side chains. Even certain differences between zein and gliadin may be worth noting. Thus the former is poorer in amide and pyrrolidine groups, whereas the latter is poorer in the number of non-polar CH_2 groups in paraffin side chains.

As compared with the six cationic and eight or ten carboxylic groups of zein, and the eighteen cationic and eighteen free carboxylic groups of gliadin, egg albumin has approximately twenty-eight cationic and an equal number of free carboxylic groups and is extremely soluble in water, being, therefore, classified as an albumin. The protein of roughly the same molecular weight, crystallized some years since from the albumin fraction of milk by Palmer,[41] has a solubility of approximately 0.8 gram per liter in water at 25° C., but is relatively soluble in salt solutions and has therefore been called lactoglobulin. Although we already know a great deal about this protein as a result of physical-chemical studies,[42] analytical studies regarding its amino acid composition remain inadequate.

Two of the other proteins of this size have well known physiological functions: pepsin is an enzyme, insulin a hormone. Pepsin is very poor in basic amino acids, rich in free carboxyl groups and has a very acid isoelectric point. It is also richer in tyrosine than the other proteins thus far considered, though not richer than insulin. Perhaps the outstanding characteristic of the latter protein, however, is the large number of cystine residues that it contains rather than its electrically charged groups, which would not appear on the basis of existing analyses to be

very different from those of other proteins. Pepsin may be classified as a globulin, since it is soluble in salt solutions but not in distilled water. Certainly there are differences in the composition of these proteins of nearly the same size and shape, but certainly also these do not yet suffice to allow us to predict behavior—especially physiological behavior.

The evidence thus far available suggests that the molecular weight in dilute aqueous salt solution of hemoglobin, of casein and of edestin are of the sizes indicated. These molecular weights are many times the minimal molecular weights calculated on the basis of composition, and it has been reported that the molecular weights of these proteins in concentrated urea solutions and also under certain other conditions are far smaller. The number of reactive groups of each kind in these proteins may most readily be compared with those of molecular weights from 34,000 to 42,000 by dividing the results for hemoglobin by two, for casein by three, and for edestin by from six to eight. Among other differences it should be noted that edestin is far richer in guanidine, and hemoglobin in the imidazole group. The imidazole group of the latter has been demonstrated to be in such a relation to the prosthetic iron containing group that its state influences the combination of the protein with oxygen.[43,44,45]

Physiological behavior may often depend upon a very small fragment of the molecule, the groups of which are arranged in a special configuration. In the case of hemoglobin and of cytochrome, of the hemocyanins, of the yellow enzymes, and of certain other proteins the reactive group may be considered as prosthetic, that is, as not being derived from amino acids. In the case of insulin, however, as we have seen, investigation has thus far revealed no components of the molecule other than the normally occurring amino acids, and the specificity of physiological behavior of this molecule,[4] as of many enzymes,[6,7] would thus appear to depend merely upon the arrangement in space of charged and uncharged, polar and non-polar side chains.

Influence of Polar and Non-polar Groups upon Solubility in Organic Solvents

Certain correlations can be made between proteins that are rich in cationic groups and in acid combining capacity, between those that are rich in anionic groups and in base combining capacity, and that are poor in ionic groups, and therefore in amphoteric properties. Those among

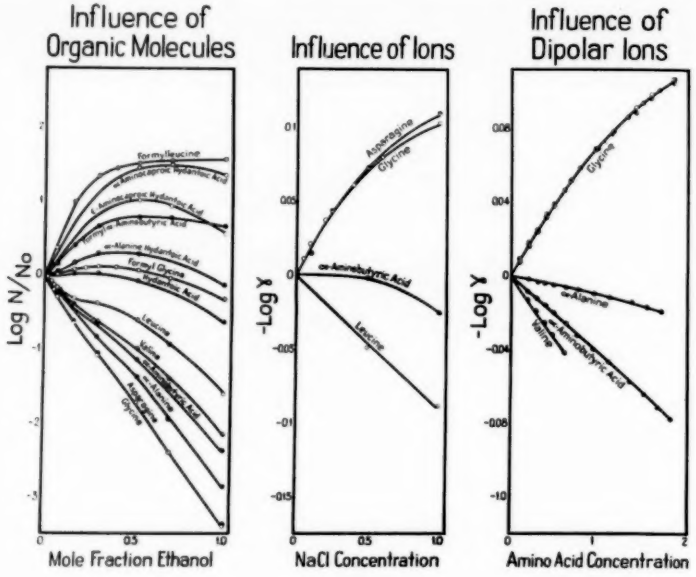

Fig. 1a Fig. 1b Fig. 1c

the latter rich in pyrrolidine and paraffin groups appear also to be more soluble in ethanol-water mixtures than in aqueous solutions and this has been the method for their isolation and the basis for their classification. It has been possible to develop a firm quantitative foundation for these generalizations by studying molecules of known structure containing the various groups of which proteins are possessed.

Glycine is the most soluble in water of the mono-amino mono-carboxylic, aliphatic α-amino acids. The additional CH_2 group of alanine renders it less soluble in water. Valine with 3 CH_2 groups in its side chain is still less soluble,[46] and the solubility of leucine with 4 such CH_2 groups is 0.0744 mole per liter as contrasted with 2.886 moles per liter for glycine at 25° C. These amino acids are all far less soluble in alcohol-water mixtures than in water, but the effect of alcohol in diminishing solubility is smaller the larger the number of CH_2 groups of which the α-amino acid is possessed. This is illustrated in Fig. 1a in which the logarithm of the solubility in any given solvent, *N*, divided by that in water, *No*, is plotted as ordinate and the mole fraction ethanol in the system as abscissa. That the smaller effect of ethanol in diminishing solu-

bility the longer the paraffin side chain depends upon the dipolar ionic nature of the neutral amino acids has been demonstrated by their comparison with certain of their derivatives that have been purified or synthesized by my colleague, T. L. Meekin.

Curves describing the behavior in ethanol-water mixtures of the formyl derivatives of the amino acids and their hydantoic acids,[47] also form a family, though their shape is quite different from that of the amino acids from which they are derived. Whereas small amounts of alcohol diminish the solubility of α-amino acids, they increase the solubility of their derivatives which are no longer dipolar ions. This is particularly marked with formylleucine and α-aminocaproic hydantoic acid. The additional CH_2 groups of these molecules as compared with hydantoic acid are reflected by increased solubility in systems rich in ethanol. The isomeric α-aminocaproic hydantoic acid, in which the CH_2 groups lie between polar groups behaves far more like the formyl derivative of α-aminobutyric acid[47]; that is to say, like a molecule with two less CH_2 groups. Its solubility in 80 per cent ethanol is approximately tenfold that in water and threefold that in ethanol, a type of behavior characteristic of the prolamines, zein and gliadin.

The influence of each CH_2 group in the transfer from water to ethanol would, however, appear to be the same for the α-amino acids or for their derivatives. This has previously been demonstrated[46] on the basis of data such as that in Table III by subtracting log N_A/N_O (where N_A is the solubility in mole fraction in ethanol) of glycine from alanine and from α-aminobutyric acid. The differences per CH_2 group are the same, not only for this comparison but for that of the other amino acids of the same family that have been adequately studied in this way, as well as for derivatives of the amino acids and for isomers of the amino acids, such as the α hydroxyamides, glycolamide and lactamide, which have the same composition though different structures than the amino acids. Moreover, expressed in these terms the effect is always positive and may tentatively be put equal to 0.5. Differently stated *each CH_2 group in side chains terminating in a methyl group may be thought of as increasing solubility in ethanol relative to that in water threefold for each CH_2 group.*

The non-polar CH_2 group does not have this effect when placed between polar groups.[47] In the peptide chain CH_2 groups alternate with CONH groups and the element CH_2CONH may therefore be con-

sidered as the repeating pattern in the protein and as having a length in the stretched condition of 3.5 Å. as revealed by x-ray studies of the fibrous proteins.[39,48]

The influence of the configuration CH_2CONH is opposite in sign to that of the CH_2 group as is demonstrated by the studies of three series of compounds in Table III. This configuration, which constitutes the backbone of the protein molecule, as well as certain protein side chains, must therefore be considered strongly polar in nature.[49]

The hydroxyl group must also be considered polar; indeed it is because of this group that the glycerols, alcohols, and water are to be regarded as polar solvents; water being the most polar since it has no CH_2 group.

The influence of the hydroxyl group in proteins on the transfer from water to ethanol is estimated in Table III from a comparison of amides and hydroxyamides, of serine and alanine and of threonine and α-amino-n-butyric acid.[49] The comparisons of hydroxyproline and proline and of tyrosine and phenylalanine are omitted, since they include the effect on non-polar carbon rings of their position between polar groups.

The sulfur of methionine is between non-polar groups, and comparison of this molecule with one of the same composition save for the sulfur suggests that the latter diminishes the influence of the paraffin side chain by an amount approximately equal to one CH_2 group. Comparison of phenylalanine with alanine however demonstrates that *the effect of the non-polar benzene ring is of the same sign as the effect of the CH_2 group, but opposite in sign to the CH_2CONH and OH groups.*

Far greater than any of these is the influence of dipolar ionic structure. The demonstration in 1923 by Bjerrum[50] that amino acids were to be regarded as zwitterions or dipolar ions rendered it possible to understand the very strongly polar nature not only of amino acids, peptides and proteins, but their far higher solubility in water than in non-polar solvents. The amino acids are compared with certain of their derivatives in Fig. 1 and in Table III. The change in log N_A/N_o may be taken as close to -2.7, whether amino acids and peptides are compared with hydantoic acids or with α-hydroxy-amides. *The influence of dipolar ionic structure upon this transfer is therefore at least four times as great as that of an hydroxyl group or of the CH_2CONH configuration.* Although the backbone of the protein may thus be conceived of as consisting of alternating polar and non-polar groups, and although many of

TABLE III

INFLUENCE OF STRUCTURE ON SOLUBILITY IN WATER AND ETHANOL AT 25°

Substance	log N_A/N_o	log N_A/N_o	$\left(\frac{\Delta \log N_A/N_o}{n}\right)$
Influence of CH_2 group			
Glycine-alanine	—3.391	—2.856	+0.54
Glycine-*a*-aminobutyric acid	—3.391	—2.375	+0.50
Glycine-*a*-aminocaproic acid	—3.391	—1.414	+0.49
Valine-leucine	—2.158	—1.622	+0.54
Hydantoic-*a*-alanine hydantoic acid	—0.630	—0.137	+0.49
Hydantoic-*a*-aminocaproic hydantoic acid	—0.630	+1.352	+0.49
Glycolamide-lactamide	—0.799	—0.254	+0.54
Glycolamide-*a*-hydroxycaproamide	—0.799	+1.084	+0.47
Formylglycine-formylaminobutyric acid	—0.330	+0.651	+0.49
Formylglycine-formylleucine	—0.330	+1.556	+0.47
Influence of CH_2CONH group			
Glycine-diglycine	—3.391	—4.367	—0.98
Glycine-triglycine	—3.391	—4.965	—0.79
Hydantoic acid-diglycine-hydantoic acid	—0.630	—1.533	—0.90
Hydantoic acid-triglycine-hydantoic acid	—0.630	—2.253	—0.81
Glycolamide-glycolylglycine amide	—0.799	—1.517	—0.72
Influence of OH group			
Acetamide-glycolamide	—0.120	—0.799	—0.68
Propionamide-lactamide	+0.016	—0.254	—0.27
Caproamide-*a*-hydroxycaproamide	+1.726	+1.084	—0.64
Alanine-serine	—2.856	—3.362	—0.51
a-Aminobutyric acid-threonine	—2.375	—3.070	—0.69
Influence of methionyl sulfur			
a-Aminovaleric acid-methionine	1.90*	—2.444	—0.54
Influence of benzene ring			
Alanine-phenylalanine	—2.856	—1.453	+1.40
Influence of dipolar ionization			
Glycine-glycolamide	—3.391	—0.799	—2.59
Alanine-lactamide	—2.856	—0.254	—2.60
Norleucine-*a*-hydroxycaproamide	—1.414	+1.084	—2.50
Glycine-hydantoic acid	—3.391	—0.630	—2.76
Alanine-alanine hydantoic acid	—2.856	—0.137	—2.72
a-Aminocaproic acid-*a*-aminocaproic hydantoic acid	—1.414	+1.352	—2.77
Diglycine-glycolylglycine amide	—4.367	—1.517	—2.85
Diglycine-diglycine hydantoic acid	—4.367	—1.533	—2.83
Triglycine-triglycine hydantoic acid	—4.965	—2.253	—2.71

* Interpolated from *a*-aminobutyric and *a*-aminocaproic acids.

the side chains of proteins are polar, the ionic groups and the dipolar ionic structure of proteins must be considered for an adequate understanding of protein behavior.

The properties of the proteins must from this point of view be conceived of as depending upon the properties, the number and the distribution of the polar and the non-polar, the ionic and the uncharged groups of which they are composed, of their volumes, their moments, their arrangements in space, and their reactivities with ions and other dipolar ions.

Influence of Polar and Non-polar Groups upon Solubility, and upon Activity Coefficients of Dipolar Ions in Salt Solutions

The precipitation of proteins by neutral salts has been employed as a method for "their separation, purification, characterization and occasionally classification"[51] ever since the "procedure was first employed in the middle of the last century by Panum,[52] Virchow[53] and Claude Bernard."[54] Hofmeister demonstrated that "salting-out" depended upon the character of the neutral salt as well as of the protein, and his studies of solubility in concentrated salt solution have since been supplemented by those of Chick and Martin,[55] Sörensen and his coworkers,[56] and by various studies from this laboratory.[51,57,58] Certain of the results in concentrated ammonium sulfate solutions are graphically represented in Fig. 2 in which the logarithm of the solubility is plotted as ordinate and the ionic strength, T/2, as abscissa. The linear relation that then obtains was demonstrated in 1925[30] and its significance discussed. This "salting-out" relation is characteristic not only of proteins but of gases, of uncharged molecules and, in sufficiently concentrated solutions, of electrolytes. The problem that remained therefore was to determine which groups, or arrangement of groups, of proteins led to their being readily precipitated, which to their being precipitated only from concentrated solutions of certain neutral salts.

On the assumption, implicit throughout this discussion, that amino acids containing the same groups are the prototypes for protein behavior, one may consider the solubility in salt solutions of these smaller molecules of known structure. Pfeiffer and his colleagues[59,60] demonstrated that the solubility of leucine was decreased with increase in sodium chloride concentration, but that the solubility of glycine and aspartic

acid was increased. Tryptophane and tyrosine[61] behave much like leucine, but the solvent action of sodium chloride upon asparagine is greater than upon glycine, whereas that of α-amino-n-butyric acid is but little influenced by low concentrations but precipitated by high concentrations of sodium chloride.

Certain of these results are graphically represented in Fig. 1b. The solubility in water of leucine is so low that solubility ratios may be considered to yield activity coefficients, but that of glycine is so high that a correction must be made for the activity coefficient of glycine in its own saturated solutions (Fig. 1c). The activity coefficient of glycine in sodium chloride, calculated from the freezing point measurements of Scatchard and Prentiss,[62] have therefore been substituted in Fig. 1b for the earlier solubility measurements.

All of the amino acids whose activity coefficients are considered in Fig. 1 are α-amino acids and therefore have the same moment due to their dipolar ionic structure. They differ from each other only in the length and nature of their side chains. The "salting-out" effect is greatest for leucine which has the largest number of non-polar CH_2 groups, and begins at low salt concentrations as is the case with such proteins as fibrinogen.[57]

α-Aminobutyric acid with a smaller paraffin side chain is precipitated only from concentrated solutions as is the case with most proteins. That the "salting-out" from aqueous solutions does not depend completely upon the volume of the molecule follows from the comparison of the solubility in salt solution of α-aminobutyric acid with asparagine and glycine. The two former have almost identical apparent molal volumes,[63] yet the activity coefficient of asparagine is slightly greater than that of glycine, presumably because of the polar nature of its terminal amide group.

Proteins like amino acids may be precipitated from aqueous solution either by organic solvents, such as alcohol and acetone, or by neutral salts, such as phosphates and sulfates. For the amino acids and presumably for the proteins, precisely the opposite groups are responsible for these effects. The order of precipitation is inverted in Figs. 1a and 1b. Molecules with side chains terminating in non-polar groups, though less soluble in the polar solvent, water, are not so readily precipitated by organic solvents as those of the same dipole moment but whose side chains terminate in polar groups. They are however more readily pre-

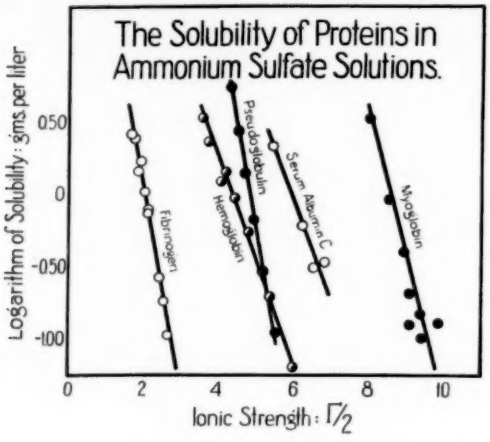

Fig. 2

cipitated by neutral salts. Conversely, molecules constituted predominantly of polar groups are more soluble in the polar solvent, water, from which they are precipitated by organic solvents but dissolved by neutral salts, especially by salts of univalent anions and multivalent cations.

Influence of Polar and Non-polar Groups upon the Activity Coefficients of Dipolar Ions

The interactions of importance in biological systems are not only those between ions and dipolar ions, but also those between the various ionic and especially dipolar ionic species present as components. The present state of experimental and of theoretical knowledge regarding interactions between dipolar ions in aqueous solution have recently been considered elsewhere.[37] When one of the dipolar ionic species is but slightly soluble, as in the case of asparagine and cystine, solubility methods have been employed in estimating the activity coefficient of one component in the presence of the other. Such knowledge has now been supplemented by freezing point and vapor pressure measurements upon amino acid solutions, and has yielded the activity coefficients of the single solutes as a function of their concentration.

The simplest dipolar ion, glycine, has been investigated both by freezing point[62] and by vapor pressure[64,65] measurements. The results are in excellent agreement and are graphically represented in Fig. 1c

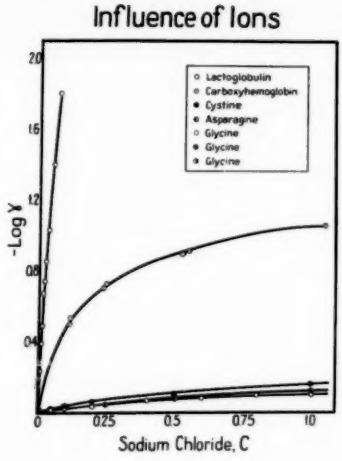

Fig. 3a

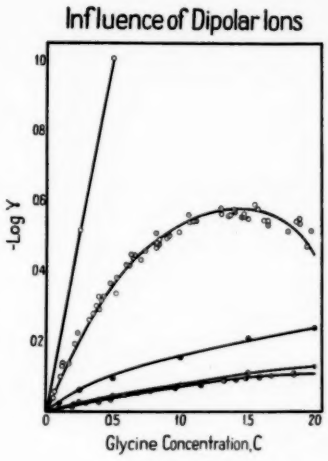

Fig. 3b

in which they are contrasted with activity coefficients calculated from vapor pressure measurements for alanine, α-amino butyric acid and valine. These results may, therefore, be compared with changes in the activity of the same components brought about by neutral salts (Fig. 1b). The comparable nature of the forces between dipolar ions and between ions and dipolar ions is evident. The influence of glycine molecules upon each other, or upon other dipolar ions, is superficially not dissimilar to that of sodium chloride (Fig. 3). *Dipolar ions with paraffin side chains, however, are "salted-out" by each other much as they are by neutral salts.*

In how far the activity coefficient of a dipolar ion will deviate from unity, in how far its interactions with other dipolar ions or with ions will lead to mutual precipitating action or mutual solvent action, will thus depend upon the balance between its non-polar groups and polar groups, especially its dipolar ionic groups.

Influence of Dipole Moment upon the Activity Coefficients of Dipolar Ions

The polarity of a group is quantitively measured only in the gaseous state or in non-polar solvents and is generally defined as a displacement of "the center of gravity" of the positive charge from that of the negative charge. The dipole moment is the product of this distance and of

the charge on the electron, 4.8×10^{-10}. The moment of the alcohols due to their hydroxyl group is 1.7×10^{-18} electrostatic units, and that of water is only slightly higher, 1.87×10^{-18}. The esters of the amino acids have been studied in benzene,[66] and the moments and volumes of the groups that we have been considering have been estimated from these and related studies. The moments of urea, amino acids, peptides and proteins are all far higher than these. Urea has a moment of 5.4×10^{-18} electrostatic units, but those of α-amino acids have been estimated to be approximately 15, of cystine 27, of diglycine 26, triglycine 32, lysyl-glutamic acid 59, of egg albumin 180, of hemoglobin 500, of lacto-globulin 700, and of pseudoglobin 1300×10^{-18} electrostatic units* on the basis of dielectric constant measurements.

In the preceding discussion only α-amino acids or their derivatives have been considered. The distance of separation between the positively charged ammonium and the negatively charged carboxyl group was therefore always closely the same and equal approximately to 3 Å. This distance of separation is, of course, greater in the peptides, and this is reflected by larger dipole moments. In the case of the proteins the magnitude of the dipole moment depends upon the spatial distribution of the ionic groups. Were the cationic groups all at one edge of the molecule and the carboxylic groups at the other, the dipole moments would be many times larger than those observed. On the other hand, were the electrically charged groups arranged with complete electrical symmetry, the molecule would have no dipole moment. All proteins thus far investigated have dipole moments, and if these are small in comparison with what they might be on the basis of the number of ionic groups, and the dimensions of proteins, they are far larger than those of any other known substances.

The interactions of peptides and neutral salts (in regions of low dielectric constant where the "salting-out" effect is small in comparison with Coulomb forces) have previously been reported and demonstrated to increase with increase in dipole moment.[70] Moreover, *the logarithm of the activity coefficient was found to increase by somewhat less than the first power of the dipole moment.*

Neutral salts have a profound solvent action upon the class of proteins termed globulins. This effect, first noted by Denis, was investigated

* The electric moments of peptides and proteins have recently been considered in detail;[21,26,28,67,68] the methods of measurement and of calculation are therefore not considered here. That of lacto-globulin is from measurements by J. D. Ferry, Cohn, Oncley and Blanchard.[69]

in 1905 by W. B. Hardy[71] and by Mellanby[72] for serum globulin, and by Osborne and Harris[73] for edestin. The changes in solubility observed are so large as to form the basis not only for classification but also for methods of purification. Presumably the dipole moments of these molecules are also very large but they have not yet been investigated, nor have preparations of these proteins been available in such a state of purity that their solubility in solution was independent of the amount present in the solid phase.

Hemoglobin and lactoglobulin satisfy this criterion as closely as any proteins thus far investigated. Activity coefficients in aqueous sodium chloride of hemoglobin[51] and lactoglobulin[41] have been calculated from solubility measurements, and are graphically represented in Fig. 3 a, where they are compared with those of glycine, asparagine and cystine. As in the case of the peptides, the logarithm of the activity coefficient increases with the dipole moment, and as a first approximation, by slightly less than the first power of the dipole moment.

These results would lead one to conclude that globulins were proteins of high dipole moment. Their low solubility in water presumably inheres, therefore, in very high crystal lattice energies for this class as for the peptides of glycine.*

The dipole moments of proteins would appear to be one of their most important characteristics, largely defining not only their interactions with neutral salts, but also with other dipolar ions. The solvent action of glycine upon hemoglobin has been investigated by Richards,[64] and upon lactoglobulin by us.[69] Activity coefficients calculated from these results are graphically represented in Fig. 3 b in which they are compared with the amino acids, glycine, asparagine and cystine. The similarity in the action of sodium chloride and of glycine is evident, both in the interactions of dipolar ions and of ions and dipolar ions, and $-\log \gamma$ would appear to increase by something less than the first power of the dipole moment.

The presence of greater numbers of paraffin side chains on the dipolar ions decreases these interactions. The interactions between ions and dipolar ions would also be smaller if, as anions, we considered phosphates, sulfates or acetates, or as cations potassium, rubidium or cesium. On the other hand, they would be greater were the anions bromides or

* The peptides of glycine are more insoluble the larger the number of glycine residues in the chain. On the other hand, for molecules such as α and β-alanine and α and ϵ-aminocaproic acids, the isomer of greater dipole moment is the more soluble when there is no difference in density in the solid state.[46]

iodides, or the cations lithium or calcium,* strontium or lanthanum.

Influence of Dielectric Constant upon the Activity Coefficients of Dipolar Ions

The dielectric constant of a solution may be conceived of as a measure of the dipole moments of the polar groups, and the number of these contained per unit of volume. The dielectric constant of a vacuum is thus unity, and that of the alcohol series is larger the smaller the number of non-polar CH_2 groups to which the polar hydroxyl group is attached. Indeed it can be shown that the product of the molal volume and the dielectric constant of solutions of the alcohols from heptanol to methanol is essentially constant[38,76] and that this relation extends also to the first member of the series, water, which has a dielectric constant of 78.54 at 25° C. as compared with 32.71 for methanol, 24.28 for ethanol and 20.83 for acetone.

But few solvents have higher dielectric constants than water. Among them are hydrocyanic acid and formamide. The latter substance is the first member of the aliphatic amide series, and is in the same relation to acetamide and proprionamide as water is to methanol and ethanol. Two of the most polar solvents known, therefore, possess this property by virtue respectively of the hydroxyl and amide groups, both of which, as we have seen, are present in large numbers as constituent parts of protein molecules.

Aqueous solutions of urea have higher dielectric constants than water, and the moment of urea has been estimated, as we have seen, to be 5.4×10^{-18} electrostatic units, or roughly, three times that of the hydroxyl group. The moment of glycine, 15×10^{-18} electrostatic units is, however, three times that of urea, cannot be ascribed to the moments of its polar groups, and is thus evidence of the dipolar ionic structure of the amino acids. The dielectric constant of solutions containing glycine is greater by 23 per mole of glycine per liter of solution. This dielectric constant increment would appear within the limits of measurement to be the same for all α-amino acids, to be greater by 13.3 for each CH_2 group separating the positively charged ammonium from the negatively charged carboxyl group, and by 46.0 for each CH_2CONH group separating the dipolar ionic groups in the series of monoamino-monocarboxylic peptides that have thus far been investigated.[38,77]

* That is assuming that calcium did not lead to a change in the molecular weight of the protein as appears to be the case for casein[74] and certain serum globulin fractions.[75]

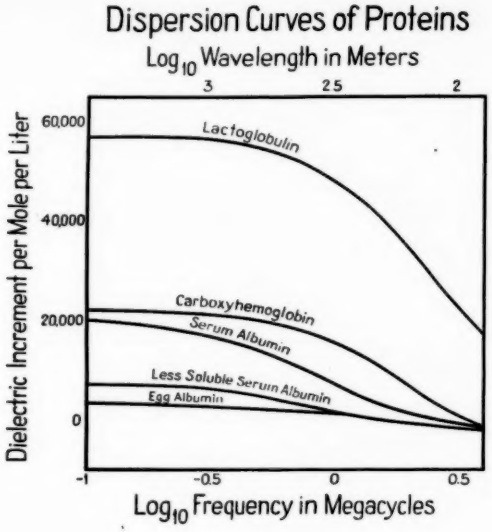

Fig. 4

Peptides have been studied which like cystine contain two positively and two negatively charged groups. The dipole moments of such molecules depend upon the spatial arrangement of the charged groups and so do their dielectric constant increments. This is equally true of the multipolar proteins whose moments have been estimated by means of dielectric constant measurements.

Methods of measuring the dielectric constants of protein solutions have elsewhere been discussed,[67,78,79] but the significant results of such measurements may well be considered. Placed in an alternating field the isoelectric protein molecules are oriented by virtue of their dipole moments and the capacity of the system and dielectric constant of the solution is thus increased. The curves in Fig. 4 represent this effect, the dielectric constant increments being given by the measurements at low frequency as 3,100 for egg albumin, 22,000 for hemoglobin and 56,000 for lactoglobulin. Of the fractions of crystallized serum albumin that have been investigated[80] the more soluble has a dielectric constant increment of nearly three times the least soluble fraction thus far investigated.

At higher frequencies the large protein molecules can no longer follow the alternating potential and dispersion of the dielectric constant

results. Plotting the measured dielectric constant against the frequency yields dispersion curves of the kind represented in Fig. 4. At sufficiently high frequencies, the dielectric constant measured is actually smaller than that of the pure solvent, and this effect will be greater the larger the volume of the protein which is no longer able to follow the current and the larger, therefore, the number of the smaller solvent molecules which would be able to follow the current at these frequencies, but which are displaced by the protein molecule.

The critical frequencies approximately represented by the midpoints of the dispersion curves are thus characteristic of the size and shape of the protein molecule, and are functionally related to the relaxation times, a quantity which presumably is of significance in electro-physiology. If proteins have different moments but the same size and shape, they will have the same relaxation time, and this would appear to be the case for all the crystalline serum albumin fractions thus far investigated. Molecules of the same size but different shape will, however, have different relaxation times, and this is true among those whose molecular weight lies between 34,000 and 42,000 of egg albumin and lactoglobulin, and among those whose molecular weight lies between 66,000 and 73,000 of hemoglobin and serum albumin. The latter is presumably the more asymmetric and therefore diffuses more slowly, has a higher viscosity and a higher relaxation time.

Measurements of dielectric constant may thus be employed in estimating not only the dipole moments of proteins, but also their relaxation times, sizes and shapes,[26,67,80,81] and may be expected to be of great value in the characterization of proteins. Moreover, the dielectric constant of a solution is one of the most important quantities in determining interactions in which electrostatic forces play a role. The high dielectric constants of protein solutions suggest that many systems of biological importance have high dielectric constants, and that an adequate understanding of interactions in certain cells and tissues demands the development of a chemistry for systems of high dielectric constant.

There is a growing body of knowledge regarding the influence upon ions of substances that increase the dielectric constant of aqueous solutions. The influence of amino acids and proteins upon insoluble salts has been explored by the method of solubility[82,83] and upon soluble salts by the freezing point method,[62,84] or the electromotive force method.[85]

Rates of reaction depend upon the activity coefficients of the reac-

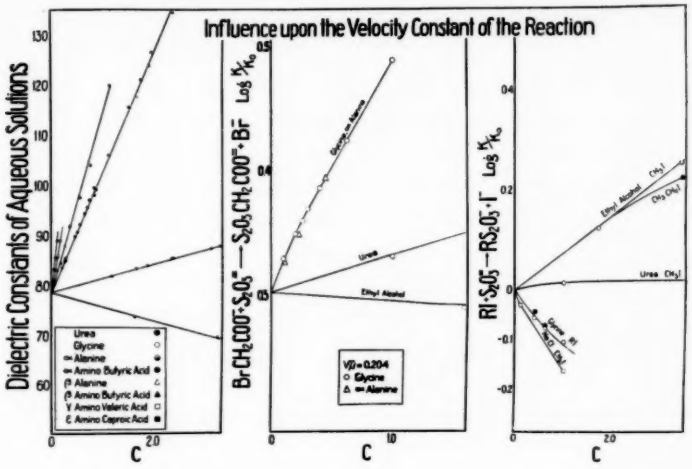

Fig. 5a Fig. 5b Fig. 5c

tants. The influence of substances which increase the dielectric constant has been explored upon the reaction of a substance (thiosulfate) on the one hand with uncharged molecules, on the other with ions.[86] The rates of reaction with the uncharged molecules, increased by ethanol and to a small extent by urea, were found to be decreased by dipolar ions much as they were by ions (Fig. 5c). On the other hand, rates of reaction with ions (Fig. 5b) were found to be increased by urea and dipolar ions as well as by ions. That the influence upon the rate of reaction was not due merely to change in dielectric constant was demonstrated, however, by the study of solutions iso-dielectric with water. The dipolar ions employed were amino acids. Those of greater molecular weight and electrical moment, such as are more characteristic of proteins, remain to be investigated, both from the point of view of their influence upon the environment, and of their role as reactants and as enzymes.

The lowered activity coefficients of ions in dilute aqueous solution have been demonstrated[87] to depend largely upon electrostatic forces. Such forces, according to Coulomb's law, are inversely proportional to the dielectric constant. In regions of high dielectric constant, therefore, Coulomb forces are diminished and activity coefficients increased. Under these circumstances forces such as those of van der Waals become large in comparison with the greatly diminished Coulomb forces, and differ-

ences in the behavior of ions of the same valence type and of dipolar ions of the same electric moment become apparent.* It is under these circumstances that the so-called Hofmeister series is observed and that great specificity of interaction may be expected.

The influence of dipolar ions upon ions and other dipolar ions in systems of many components will thus be to raise the dielectric constant and thereby to diminish electrostatic forces. Systems containing ions and two dipolar ionic species are the most complex that have thus far been investigated,[88] but studies upon systems which approach still more closely to those that obtain in nature are in progress, and there would appear to be no differences in principle which should make it impossible to pass from the investigations that have been carried out thus far to the study of interactions between the components of the cells and tissues of the body.

Summary

In order to gain insight into the behavior of even the simplest proteins, we have thus been forced to explore the behavior of molecules of known structure such as the amino acids and peptides. Only those proteins have been considered in this discussion which have been isolated in a relatively pure state, and could therefore be investigated as chemical substances. The methods would appear to be at hand, however, for the study of all proteins. There is no theoretical obstacle to the isolation of all the protein constituents of any given tissue, or to their characterization as chemical substances, and to the study of their interactions as biological components. Proceeding thus, often with new techniques, but employing the classical methods of physical chemistry, we may hope in time to achieve an understanding of the morphology and physiology of biological systems in terms of the properties of their components.

The description of the proteins of tissues as biological components of known size and shape, the number and distribution of their free groups apprehended and the mechanism of their interactions described remain largely to be accomplished. These will depend in many cases upon the spatial configuration of the free groups of the amino acids of

* Conversely, the lower the dielectric constant the greater the Coulomb forces in comparison with the salting-out effect and other non-Coulomb forces. Under these conditions, considered elsewhere,[89] the principle of the ionic strength obtains in the interactions between ions and dipolar ions, and the valence of the former, and the electrical moments of the latter determine behavior.

which they are composed, and in others upon the presence of more specialized prosthetic groups.

The properties of the proteins as a class permit certain generalizations to be made. By virtue of their large size proteins are retained by cell walls through which oxygen and carbon dioxide, urea and lactic acid, amino acids and many salts freely pass.

By virtue of their ionic nature proteins form salts with each other and with smaller organic and inorganic acids and bases. Certain of these are completely dissociated, others have been judged to be undissociated on the basis of freedom of the non-protein ion to diffuse or to give some chemical test. The precise nature of most of these interactions remains to be investigated.

By virtue of their dipolar ionic structure proteins have high electric moments. These lead, as we have seen, not only to dipole-dipole interactions, but to formation of dipole pairs and to aggregates containing larger numbers of molecules, often arranged with spatial symmetry with respect to each other and dependent upon the shapes of the molecules, the number and distance from the edge of their electrically charged groups and the resulting dipole and multipole moments. As a limiting case the electric moments in the solid state lead to high crystal lattice energies not only in the packing of chemical individuals but of mixed crystals.

By virtue of their high dipole moments proteins contribute high dielectric constants to their solutions. In environments of high dielectric constant Coulomb forces are reduced and conditions obtain that differ widely even from those characteristic of dilute aqueous electrolyte solutions. Although completely dissociated, electrostatic interaction is so reduced that the principle of the ionic strength no longer determines behavior. Under these circumstances, differences in size and shape as well as in valence must be taken into account and non-electrostatic, especially van der Waals forces, acquire primary significance. Under these conditions the behavior of glycine is readily distinguished from that of alanine and of sodium from that of potassium.

By virtue of their high dielectric constants the activity coefficients of both ions and dipolar ions are generally greater than they would be in dilute aqueous solution. As a consequence laws of ideal solution may more often be found to obtain in concentrated biological systems than in dilute aqueous solution. Since rates of reaction are dependent upon

the activity coefficients of the reactants, the high dielectric constant of many biological systems may be expected to play an important role in the kinetics of biological systems.

Various laws in terms of which interionic and intermolecular actions may be described are recent contributions of physical chemistry. Many of them are applicable to biological systems in terms of the parameters that have been discussed. It remains to describe in far greater detail the characteristics of the proteins of biological systems as chemical substances and approach ever more closely to the conditions that obtain in biological systems, and which in large part depend upon the highly specialized physical-chemical characteristics of the protein molecule.

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ON THE ORIGIN AND DEVELOPMENTAL POTENTIALITIES OF BLOOD CELLS*

CHARLES A. DOAN

THE nature and variety of the functions performed by the cells of the blood, in health and in disease, make the problems concerning their origin, development, interrelationship, and ultimate disposition of fundamental interest to medical investigators and of vital importance to the clinician. The blood elements are influenced by, and in turn affect every normal physiologic as well as every abnormal pathologic reaction. For this reason the recording of the blood cells has long since become a universal routine in clinical medicine; but this very fact has tended frequently to minimize the value and limit the interpretations which can and should be made from such data—familiarity, and careless or inexpert technique tending perhaps to breed a certain contempt for the diagnostic and prognostic value of this information. G. Lovell Gulland,[1] however, in his 1930 Harveian Oration on the Circulating Fluid, prophesied "the time when the differential count will be more important than the auscultation of the heart." For some, at least, this time has already arrived.

While Neumann first described in 1868 hemocytogenesis in the bone marrow, significant interest and effective progress in hematology dates only from 1891, when Ehrlich reported the differential tinctorial reaction of the cells of the blood to specific dyes. There followed a quarter century of intensive effort to establish working hypotheses from the new morphologic criteria thus made available, which resulted in the emergence of two broad, general, divergent and controversial interpretations: the monophyletic and the polyphyletic doctrines, with many individual modifications (Fig. 1). Embryologic studies only contributed to the confusion, and there gradually arose an intricate and extensive descriptive nomenclature, based upon minutiae of morphologic detail relating to dead cells, from which the average medical student and practitioner has not yet recovered. With the introduction, however, of the methods and

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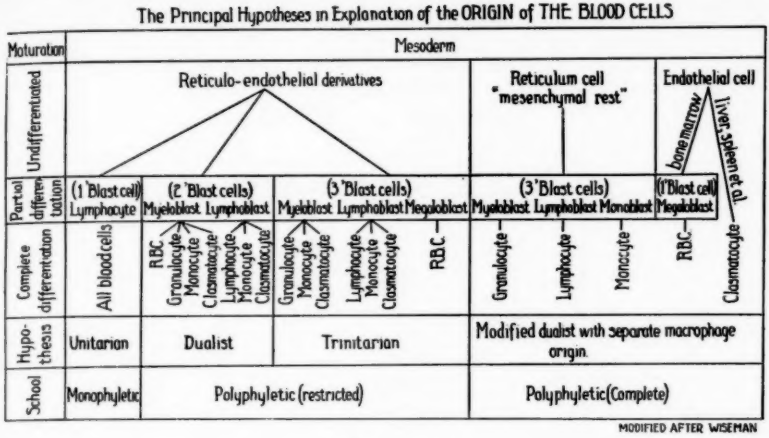

Fig. 1

tools of modern experimental physiology, including the cultivation and study of living explanted tissue *in vitro* by Harrison (1913), the development of the technique for observing hemocytogenesis in the living chick blastoderm by Sabin[2] (1920), the application of certain non-toxic vital and supravital dyes to the study of living cells by Goldmann (1909-12), Evans and Schulemann (1914-15), Simpson (1922) and Sabin (1923), the *in vitro* determination of the respiratory quotients of cells under varying conditions by Warburg (1930), the controlled nutritional studies of Whipple in dogs (1920), and the inauguration of basic clinical investigations of the human anemic and leukemic states—with the perfection of these procedures, hematologists began to accumulate a fund of dynamic data, which quite naturally has supplemented, modified and clarified the more limited morphologic speculations of the past.

In this particular domain of medicine, it is especially important to temper interpretations based upon morphology with pertinent functional observations. Throughout the mammalian life span, the cells of blood and connective tissues arise as primitive, undifferentiated, mesenchymal elements requiring a period of complex maturation, with almost infinite potential, and actual morphologic variation before the definitive units become functionally available to the body. These maturative phenomena have proved to be fertile soil for the multiplication of exceedingly involved hematopoietic hypotheses, far more complicated in morphologic

fancy than the more recently accumulated physiologic facts would seem to justify. I shall, therefore, devote little or no time either to attacking or defending any system of terminology, or in discussing the purely morphologic basis for cell classification. Rather, I shall invite your attention to certain broad, general principles of blood cell growth and blood cell responses, an understanding of which should permit each physician to envisage for himself the underlying hematopoietic and tissue reactions in disease, on the basis of currently obtainable data from the newer types of blood cell *studies* (not "counts").

Five fundamental questions, peculiar to and inherent in hematopoietic tissue, have formed the focus for all of the more recent physiologic studies of the phenomena involving the blood cells: (1) the nature and significance of cell origins; (2) the functional specificity and interrelationships of each definitive cell type; (3) the factors essential for differentiation and cell maturation; (4) the forces governing cell delivery and distribution; (5) the conditions influencing cell destruction.

Embryologic Hematopoiesis

Embryologists are in general agreement that the blood cells derive originally from the mesodermal rather than the ectodermal or entodermal cell layers (Fig. 2). It is also now accepted that the first blood cells to form in the embryo are the red cells, and that they arise intravascularly. Danchakoff[3] and Maximow[4] both affirmed this in 1908 and 1909 using fixed tissues. Sabin[2] in 1920 actually observed in continuous studies of the living chick blastoderm of the second day of incubation, mesoderm differentiating into angioblasts, which in turn gave origin to the first hemoglobin synthesizing cells, the earliest endothelium, and the first blood plasma; megaloblasts, thereafter, arose from and developed within the vascular endothelium of the area vasculosa.

Knoll[5] (1927-29) in a series of thirty-nine human embryos, obtained also in the living state and studied for the development of the hematopoietic tissues, found that the blood contained only red cells through the second month of gestation. Granulocytes appeared about two and one-half months after the red cells, and the lymphocytes still later. Only by the third month were white cells to be seen in appreciable numbers in these human embryos. During the third month the formation of the blood cells is taken over by the liver. By the latter part of the third and during

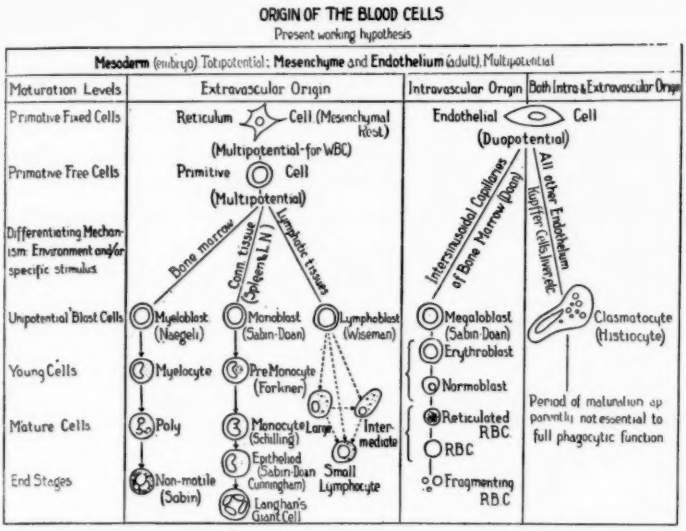

Fig. 2

the fourth month changes begin in the cartilage cells in the center of the shaft of the long bones. Vessels from the perichondrium penetrate into the zone of softening cartilage, carrying along connective tissue elements. The primitive mesenchymal cells of the marrow at this stage were accurately described and beautifully illustrated by Maximow[6] in 1910.

Bone Marrow as an Organ

In the hypoplastic areas of adult human marrow, this same simple organization of the marrow parenchyma is maintained and when analyzed, the tissue may be seen to be composed only of fat cells, vascular endothelium, and reticulum, i.e. mesenchymal, cells. Such a marrow provides the starting point, as Peabody[7] reëmphasized in 1926, for approaching an understanding of cell origins and cell relationships in the more densely hyperplastic areas of active red marrow. According to one school of hematologic thought, as the need for increased red blood cells arises, the fat cells in a quiescent sector are rapidly demobilized, the endothelium lining the intersinusoidal capillaries hypertrophies and divides, with the intravascular appearance of megaloblasts; subsequently these basophilic cells with vesicular nuclei and large single nucleoli, multiply as

they gradually elaborate hemoglobin and eventually form the typical scattered, circumscribed intravascular islands of erythroblasts and normoblasts so characteristic of "red" marrow. Full maturity embodies loss of pyknotic nucleus coincident with development of complete complement of hemoglobin and cell delivery through recanalization of the non-patent erythrogenic capillary. If the need is for granulocytes and thrombocytes, the mesenchymal "rests" (reticulum cells) show increased mitoses with the appearance of myeloblasts and megakaryocytes forming the center of extravascularly located foci of myeloid multiplication and maturation.

The Lymphocyte Question

Another interpretation of such hematopoietic activity attributes to one, common, free, stem cell, a so-called lymphocyte, multi- or totipotentialities for all other definitive blood elements. The principal division of opinion at the present time would seem to hinge upon the conception, or the definition, of a "lymphocyte." Probably no amount of discussion, based solely upon morphologic detail, can ever effectively settle the points at issue. There are none of the more distinctive characteristics, such as hemoglobin, specific granules, or patterned vacuoles to differentiate sharply the so-called small circulating lymphocyte of normal blood from other simple, undifferentiated mononuclear elements. Moreover, certain it is that each definitive strain of blood cells originates from a simple, relatively less differentiated, basophilic mononuclear cell, and only gradually evolves or elaborates its distinguishing cytoplasmic and nuclear characteristics. The farther back in the life cycle of any blood or connective tissue cell the hematologic investigator goes, the harder it is to differentiate on morphological grounds alone. Indirect, as well as direct, means must often be utilized in ascertaining essential identifying data. Pappenheim's original concept of a common stem cell, the lymphoidocyte, for all the blood cells, while receiving frequent reaffirmation (Maximow,[4] Danchakoff,[3] Weidenreich, Jordan,[8] Ferrata, Latta and Ehlers, Bloom, Lewis and Lewis) has also been frequently challenged (Ehrlich, Naegeli, Schridde, Schilling, Krumbhaar, Sabin, Cunningham and Doan, Peabody, Clark and Clark, Seeman, Tischtschenko, Hall and Furth). Jordan and his associates in a series of studies (1920-1936) of hematopoiesis in the frog, conclude that the differentiation of lymphocytes originating from reticulum cells gives rise to all of the various types of definitive blood cells. Tischtschenko[9], on the other hand, working in

His' laboratory in Berlin (1931) concludes that the myeloid and lymphoid elements in frog's blood "must belong unconditionally to two different hemocytogenic systems." Bloom, at the University of Chicago, has cultured the cells from thoracic duct lymph and observed the transformation of large and small lymphocytes into inflammatory polyblasts[10] (1928) and granulocytes[11] (1937). The Clarks[12] (1930) at the University of Pennsylvania, and Seemann[13] (1930) working under Aschoff's direction at the Pathologic Institute of the University of Freiburg, have been unable to interpret in this way their observations; and Seemann, after an extensive cytologic survey in the rat, reports that "the real lymphocyte from lymphatic tissues is not capable of transformation into monocytes and histiocytes," and states, furthermore, that the "extreme monophyletic school of Maximow places under the category of lymphocytes entirely different forms of cells, which are *only separated by supravital staining or biological experiment*." This was the conclusion of Sabin, Cunningham and Doan[14] earlier (1922-1925) working at Johns Hopkins with the supravital technique and *in vivo* animal experimental procedures. These investigators defined and differentiated the life cycle of each of the definitive strains of blood cells, ascribing to the specific endothelium lining the intersinusoidal capillaries in marrow the intravascular origin and development of the megaloblast and its progeny; to the extravascular mesenchymal reticulum, the "primitive free cell," lymphocyte-like in size and superficial characteristics, from which the myeloblast, lymphoblast and monoblast derive under appropriate conditions. An extended and comprehensive series of experiments followed at Harvard and at the Rockefeller Institute, correlating cell form with cell function under both physiologic and selected pathological conditions, which included the significant studies of Wiseman[15], all tending to establish the specificity of the life cycle and the functional independence of the so-called small lymphocyte of blood and lymph nodes. Hall and Furth[16] (1938) using thoracic duct lymph from normal dogs, and from normal, tuberculous, and B. monocytogenes-infected rabbits, found no evidence in extensive tissue culture studies of any transformation of lymphocytes into monocytes or fibroblasts. Occasional monocytes (0.1 to 2.3 per cent) as well as small (85 to 90 per cent), intermediate, and large lymphocytes (5 to 13 per cent) were encountered in the thoracic duct lymph collected for culture. In our own laboratory for the past several years, Dr. Houghton has been culturing the lymphocytes obtained from pa-

tients with lymphatic leukemia, leuko-lymphosarcoma, and infectious mononucleosis, among materials from other sources, and there has been no evidence adduced in any single instance which could be interpreted as representing a true differentiation of the type of cell recognized morphologically, metabolically and culturally as the "lymphocyte." Rather has the evidence continued to accrue in support of the concept of the functional importance of the lymphocyte *per se* in the body economy as a definitive unit, which frequently serves to antagonize, or reciprocate with, rather than evolve into other cell types.

It would seem, therefore, that the various points of view which have been expressed about the lymphocyte might best be amalgamated and integrated through the acceptance of at least two components as comprising the "lymphocyte" of the monophyletic school: the one, the classical small lymphocyte of blood, lymph nodes and spleen which rarely if ever changes its fundamental form; and the other a comparably small lymphocyte-like, 'blast cell which may always be found associated with active white cell differentiation and maturation anywhere.

Appropriating the universally accepted morphologic criteria of cell maturation common to the better known hemocytogenic cycles, viz., decreasing cytoplasmic basophilia, changes in number, form and distribution of mitochondria, disappearance of nucleoli with progressive elaboration of nuclear chromatin and cell motility, Wiseman[17] divided arbitrarily the circulating blood lymphocytes into three morphologic groups; designated them Young, Mature, and Old; and established their mean frequency and relative proportionate occurrence under conditions of health in animal and human subjects. In normal human adults quantitative values for lymphocytes were observed to range from 750 to 3,000 per c.mm., the *qualitative* formula in the average healthy individual being far more constant: Y : M : O : : 4 : 48 : 48. Thus, although the number of circulating lymphocytes fluctuates widely in health from person to person and in the same individual from time to time, the proportionate age relationships, based upon the morphologic characteristics cited, remain much more constant and have seemed to change significantly only when disease intervenes. From the evidence available, Wiseman has assumed that the absolute number of lymphocytes present in the peripheral blood at any one moment is the resultant of the balance between four major forces: (1) the speed and efficiency of differentiation and maturation of lymphocytes within the follicles of the lymph

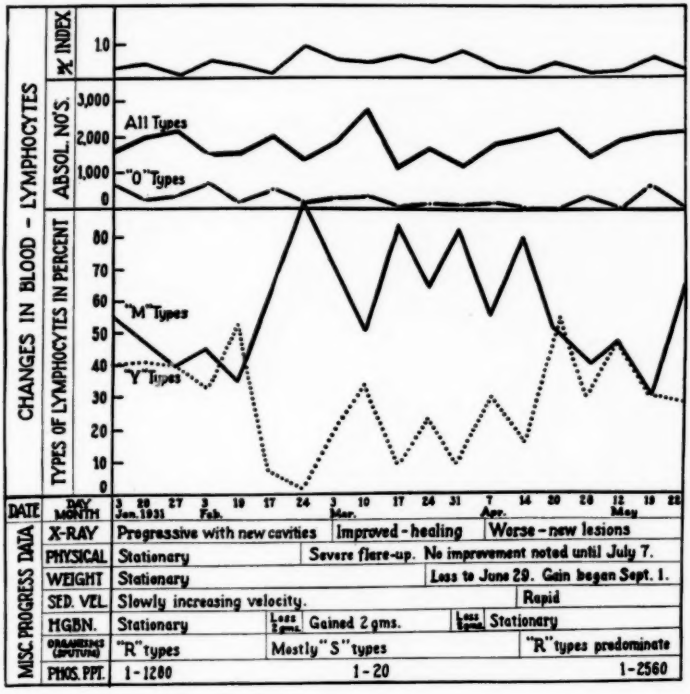

Fig. 3

W. I., white male, aged 22 years, with known clinical tuberculosis of 3 years duration, and a sanatorium history of 11 months. X-ray diagnosis: far advanced active tuberculosis with multiple cavitation. The clinical and laboratory findings are recorded. The Y:M:O qualitative relationships within the lymphocyte strain of cells changed markedly during the 5 months of observation, reflecting more sensitively than any other single criterion, the patient's reaction to his disease.

nodes; (2) the rate of delivery of these cells to the circulation; (3) the rapidity of their withdrawal or destruction, reflecting changing functional demands or toxic influence; (4) the capacity for reservoir storage of lymphocytes in the tissues. It is entirely conceivable, and in practice it has been found to occur, that a profound imbalance in the lymphopoietic equilibrium may occur without alterations in the total circulating lymphocytes, beyond the rather wide limits established for normal individuals, quite as has been recognized for the neutrophilic granulocytes since the analyses of Arneth, Schilling, and a host of subsequent observers. This relative age-maturity interpretation of the finer differentiating qualitative criteria observed in fixed and supravitally stained films of

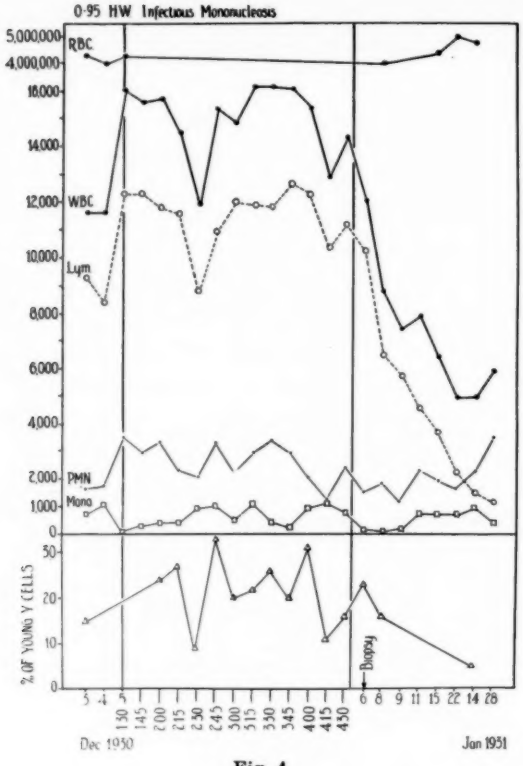

Fig. 4

The young, relatively large, lymphocyte with Reider nucleus, which usually dominates the blood picture in infectious mononucleosis, is pathognomonic of this benign dyscrasia. Serial, 15 minute interval counts were made during the period indicated on Dec. 5 to demonstrate the physiologic correlation (non-leukemic) between the fluctuations in the total lymphocyte count and the corresponding proportion of "young" lymphocytes. As the disease subsided and the lymphocytes returned to normal in number and quality, the neutrophils, which had been moderately depressed, returned to their normal number and relationship in the differential count.

normal blood could, of course, only be established and then applied clinically by submitting the hypothesis to experimental and practical tests under known conditions. This Wiseman has proceeded to do. In rabbits injected with foreign proteins or inoculated with bovine tubercle bacilli the profound disturbances in lymphocytes—reciprocal to granulocytes in the former instance and to monocytes in the latter—were only fully understood and could only be adequately correlated with the final tissue studies by recognizing and interpreting the qualita-

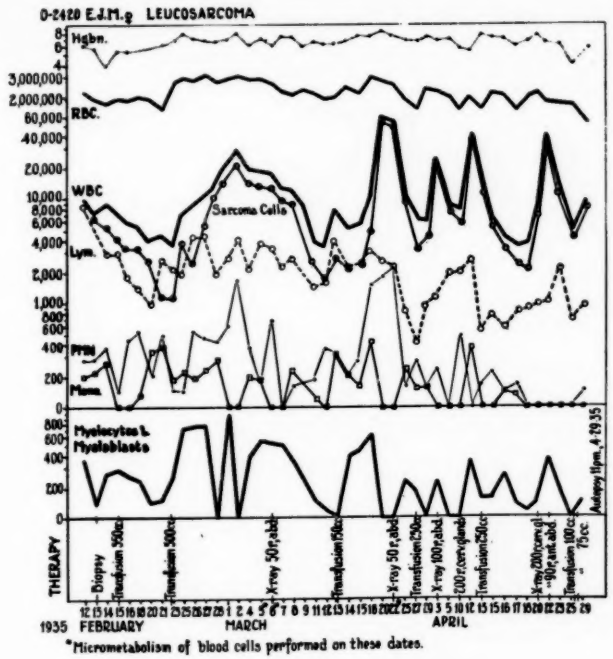

Fig. 5

In leukosarcoma, two distinct types of lymphocytes may be seen side by side in the same microscopic field; the one, normal mature, according to every criterion; the other, obviously a much younger form, with large single nucleolus and characteristic cytoplasmic changes when observed with supravital stains. Deep x-ray therapy in very small dosage destroys promptly the "sarcoma" lymphocytes, while depressing little, if any, the normal lymphocytes. A myelophthisic blood picture, with anemia, nucleated red cells, myeloblasts and myelocytes, reflects the hyperplasia of lymphosarcoma cells in the bone marrow. Micrometabolic studies have shown a malignant type of respiration for the "sarcoma" cells, in sharp contrast to the data obtained from normal and leukemic lymphocytes.

tive, in addition to any quantitative changes in the lymphocytes. In human tuberculous patients (Fig. 3),[18] in infectious mononucleosis, (Fig. 4), in leuko-lymphosarcoma (Fig. 5), in chronic lymphatic leukemia (Fig. 9),[19] the importance, significance and validity of the qualitative differentiation of the lymphocytes has been clearly established. The interpretations based upon these morphologic criteria in the circulating lymphocytes have been validated by and correlated with basal and cell metabolic studies, specific cell motility characteristics, by observed variations in cell behavior in tissue culture, and by contrasting biopsy and postmortem tissue. That is to say, the human blood lymphocyte shows a

variety of characteristic cellular alterations more or less pathognomonic of various disease mechanisms, but always it retains sufficient criteria to identify it with its own definitive maturation cycle.

The Granulocytes

Turning now to the granulocyte, a much more readily recognized maturation cycle may be seen in normal bone marrow. The agranular myeloblast has a less deeply basophilic cytoplasm than the germinal center lymphoblast, fewer and finer mitochondria, and a nucleus usually with more nucleoli. Seldom need the trained cytologist hesitate in differentiating lymphoblast and myeloblast. The more primitive free cell common to lymph node, bone marrow, and connective tissues[14] and only observed under conditions of excessive stimulation in one or other of these areas, is the cell, if any, which will be confused with the small lymphocyte of suspected multipotential capacities of differentiation. As soon as the first specific granules appear in the myeloblast—neutrophilic, eosinophilic or basophilic—the respective definitive cell type may be predicted. Coincident with granule appearance, but not before, neutrophilic and eosinophilic myelocytes become oxidase reactive, the amount of the peroxidase precipitate being directly proportional quantitatively to the number and size of the granules present in any given cell[20]; basophil granules remain oxidase negative throughout the basophil granulocyte life cycle. Lymphocytes throughout their life cycle and in their various pathologic responses, and all "primitive" and 'blast blood cells remain oxidase negative. All myelocytes lack intrinsic motility until maturity under normal circumstances, which is to say, from the morphological standpoint, until a full complement of specific granules has been developed, the basophilia and mitochondria of the cytoplasm have gradually diminished to the vanishing point and the nucleoli have disappeared and the chromatin greatly condensed in a nucleus which is beginning to constrict preparatory to lobulation. In acute infections and under certain other pathologic conditions, motile, granule-deficient neutrophilic leukocytes with mitochondria and basophilic cytoplasm, may be encountered. Throughout the myelocytic phase of maturation, the cytoplasmic criteria are the more dependable, especially granule development; less than ten specific granules per cell being designated myelocyte A, approximately 50 per cent granules, myelocyte B, and a full complement of granules, myelocyte C.[21] During this inverse reciprocal evolution in

mitochondria, cytoplasmic basophilia and specific granule formation, the nucleus becomes smaller, more pyknotic and finally segments. Ameba-like motility ensues with a liquefaction of the hitherto gel-like cytoplasm, and the relative age of the motile mature granulocyte is thereafter gauged by the number of lobes to the nucleus (Arneth[22]). The origin of the myelocytes being extravascular in the parenchyma of the bone marrow in the normal human adult, and the function of the mature granulocytes being accomplished in remote tissues or along the mucous surfaces of the body, intrinsic cellular motility is a prerequisite to the entrance of the leukocytes into, and their egress from the blood stream, except on occasions secondary to pathologic marrow hyperplasia.

The Monocyte-Clasmatocyte Relationship

The monocyte or "transitional" of Ehrlich has been almost as difficult a cell to accept and to assign specificity and independence as the lymphocyte. First, its azurophilic granules in a mottled basophilic cytoplasm with oval or slightly indented vesicular nucleus were thought to represent an earlier stage in the maturation of the myelocyte, later the azur granules of the lymphocytes were thought to be identical with them.[23] In 1913 Schilling[24] first declared that the criteria were sufficient to establish the monocyte as an independent and distinct cell entity, and the development and application of the supravital staining technique to this problem of cytologic specificity subsequently has fully supported the rightness of Schilling's conclusions. A cytoplasmic system of vacuoles was revealed in the living cell, which stains characteristically with neutral red, is arranged as a rosette opposite the *hof* of the nucleus, and is surrounded by mitochondria, all of which methyl alcohol obliterates from the ordinary fixed stained blood films. Furthermore, a characteristic surface film type of motility differentiates the monocyte in fresh preparations from both granulocyte (ameboid) and lymphocyte (peristaltic). Here again, a careful study of the peripheral nodes[25] and spleen,[26] where monocytes arise normally, and more especially the analysis of experimental monocytoses[27] establishes a maturation sequence from primitive, basophilic, non-vacuolar, non-motile, monoblast through succeeding stages of nuclear and cytoplasmic development to characteristic mature, motile monocytes. Under pathological conditions, such as tuberculosis, the monocyte may be altered by the bacterial lipids[28] to become the typical epithelioid cell and Langhan's giant cell of the tissue tubercle.

Now, morphologically and functionally distinct from the other circulating leukocytes by common consent, the monocyte still must share the connective tissue role of phagocytosis and thereby merge its identity, in the opinion of some, with the tissue macrophage or clasmatocyte. Whether these functionally and histologically similar, but not morphologically identical, cells represent different phases in the life cycle of the same cell, or whether there are two tissue phagocytes of different origin remains today a matter of debate and further experimentation. The evidence from supravital studies of experimental and pathological tissues favors the independent origin and separate identity of monocyte and clasmatocyte (Fig. 2), though recognizing at times their common response and similar behavior in non-specific tissue reactions. The clasmatocyte is the one cell of the tissues, and/or rarely of the blood, which does not seem to require a period of maturation for full functional activity.

So long as abstract static morphologic studies alone were made of the complex medley of multiple cell types, each with its own complicated life cycle, little progress of functional or clinical significance was possible; only unverifiable speculation existed. However, with the acquisition of increasingly distinctive criteria of type and age specificity, and with the means and the interest to analyze and interpret a variety of induced and spontaneously occurring cellular *disequilibria* in terms of functional responsiveness and relative tissue adequacy, substantial physiologic information of great significance has become available, and facts are slowly beginning to fortify or undermine fancies, as the case may be. With the newer knowledge, of fundamental mechanisms and inhibiting and stimulating influences upon blood cell multiplication and maturation, the formulation and application of rational prophylactic, as well as therapeutic regimes, has become possible.

Erythropoiesis

In no area of specific or general health is this more true than that of the oxygen-carrying mammalian red blood cell. Other than a relative degree of regional anoxemia, we do not yet know the additional fundamental requisites for the primary initiation of erythrogenic differentiation. When we do, the answer to some of the "primary" aplasias will probably be known. Many toxic substances will inhibit or destroy normal erythropoiesis, such as benzol, arsphenamin, x-ray, and radium;

and when promptly recognized and eliminated, recovery ensues. A congenital accentuation of physiologic splenic hemolysis may disturb the hemolytopoietic equilibrium to the point of acute hemoclastic crisis; the bone marrow is hyperplastic, usually at the late erythroblast and normoblast level of maturation, and successful splenectomy initiates a prompt erythrocyte reëquilibration, with complete clinical recovery.[29] In iron deficiency states, a hypochromic, microcytic anemia, with low plasma iron,[30] without intrinsic marrow defect may be remedied specifically through adequate replenishment of the body's depleted iron reserves. In such an instance no diminution in number of circulating or marrow red cells need exist, but iron must be available for the synthesis of hemoglobin. Vitamin C and thyroxin are also essential to the maintenance of normal erythropoietic equilibrium. When maturation arrest occurs at the megaloblastic level of red cell differentiation, an hyperplasia develops in the marrow with progressive peripheral anemia, hyperchromic and macrocytic in type. The life cycle of the erythrocyte is best observed while following the recovery from such a state (Fig. 6). Because of inability to utilize iron without the erythrocyte maturation factor, plasma or transport iron values are higher than normal.[30] Coincident with the supplying of this deficit by any one of a number of oral or parenteral sources of the active principle, iron once more is utilized in considerable quantities, reflected by a precipitous fall in the plasma iron level in direct relationship to the maturation, and, therefore, prompt disappearance of the megaloblasts as such from the marrow; in their place appears a transitory increase in early erythroblasts to be followed promptly by late erythroblasts with still more iron containing hemoglobin until, finally, by the fifth or sixth day, normoblasts dominate numerically the age-range of the nucleated red cells in the marrow with only a minimal number of the less mature erythroid elements still present. In the peripheral blood the reticulocytes usually reach their peak some 24 to 48 hours after the normoblasts have become the predominating red cell in the marrow, representing the youngest of the enucleated red cells in the circulation, and marking the culmination of the suddenly reëstablished maturation cycle. As erythrocytic equilibrium is again approached, the reticulocytes fall to their physiologic level of less than 1 per cent, and the marrow gradually resumes its normal cellular relationships, permitting the myelophthisically depressed platelets and granulocytes to regain their accustomed levels. In the patient

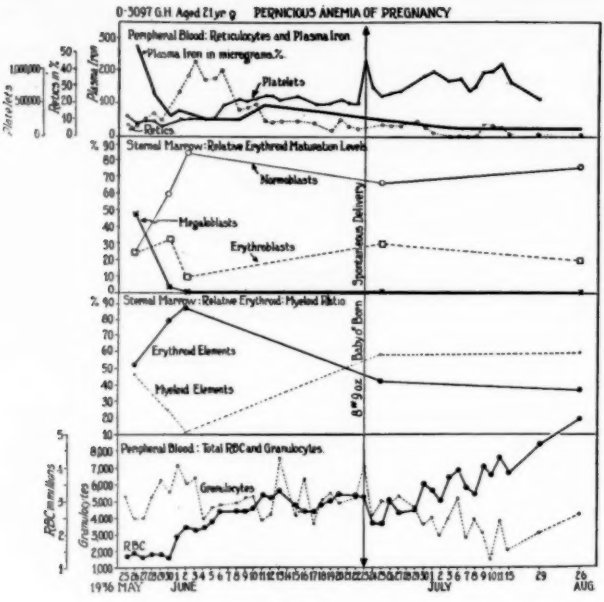

Fig. 6

"Extrinsic factor," dietary deficiency during the fifth successive pregnancy in as many years, resulted in a macrocytic anemia in this patient indistinguishable, as to peripheral blood picture, hyperplastic megaloblastic marrow and high plasma iron, from Addisonian pernicious anemia. Gastric analysis revealed 68° free HCL. The institution of a high caloric, protein diet plus 50 gms. autolyzed yeast daily brought about a prompt utilization of the excessive circulating plasma iron; this was reflected immediately in the bone marrow by increased hemoglobin synthesis, which reduced the proportion of megaloblasts; the resulting erythroblasts in turn gave way to normoblasts, to be followed by the usual reticulocyte peak in the peripheral blood. With the reduction in marrow erythroid hyperplasia, platelets and granulocytes returned to their normal levels, and, with the reestablishment of a normal red cell and hemoglobin equilibrium, plasma iron and reticulocytes became normal, and the erythroid : myeloid ratio in the marrow became physiological. An identical maturation sequence has been observed in repeated instances of adequately treated primary pernicious anemia with "intrinsic factor" deficit.

illustrated in Fig. 6, the approximate length of time for maturation from megaloblast to non-reticulated mature red blood cell might be estimated at 15 days, on the basis of the available data. Jones[31] has described the megaloblast and its interrupted maturation in Addisonian pernicious anemia as a distinctly abnormal pathologic phenomenon, bearing no relationship to, and having no counterpart in, embryologic or normal adult erythropoiesis. This has not been the interpretation of Dr. Sabin and her associates, who rather consider the macrocytic deficiency anemias to represent the result of a metabolic deficit compar-

able to the simple iron deficiency states, in which no inherent bone marrow pathology as such exists. The functional inadequacy of marrow is secondary to gastrointestinal or liver pathology; the last-named assumes primary etiologic significance in the light of Castle's, Whipple's and Minot's classical observations. Given suitable erythrocyte maturation factor, an entirely normal bone marrow, histologically and functionally, results promptly.[32]

Hemocytologic Reciprocities

Although many of the same noxious agents which depress erythropoiesis may inhibit or destroy granulocytes and lymphocytes and interfere with thrombocytopoiesis, it has become increasingly apparent that highly specific effects, either stimulatory or inhibitory, may be limited solely or largely to one cell strain. There is also evidence that significant reciprocal cell-strain relationships frequently result as a part of pathologic reactions.[33] The simplest and most readily understood example of marrow cell reciprocity is that just cited of the mechanical limitation of myelopoiesis and thrombocytopoiesis in pernicious anemia in relapse secondary to megaloblastic hyperplasia. The same diminution in normal number of circulating blood elements, with characteristic "left shift" qualitative alterations in the cells, is seen in multiple myeloma, metastatic carcinoma, osteosclerosis, leukosarcoma, the leukemias, or in any condition where such an abnormal appropriation of marrow parenchyma restricts the origin and development of normal hemopoietic foci. The remedy for specific blood cell insufficiencies resulting from these causes, lies obviously in restricting the abnormal cell growth and not in the institution of hemopoietic therapeusis *per se*.

However, more subtle reciprocal relationships among the blood cells have been observed which have a direct bearing upon the question of cell origins and their developmental potentialities and specificities. From the experimental angle it has been found that the amphophilic (neutrophilic) granulocytes in rabbits may be strongly stimulated by the nucleic acid derivatives and their components, the nucleotides, specifically, adenine and guanine.[34] A general myelocytic marrow hyperplasia resulted, and under prolonged stimulation ectopic foci of myelocytes were found in spleen and kidneys.[35] It was noted during these studies that the total number of circulating lymphocytes decreased as the granulocytes rose, and at postmortem the lymph nodes, spleen and

other sites of lymphoid development were found to be practically devoid of all follicles and all germinal center activity.[33] No myelopoietic activity and no myelocytic infiltration had occurred in these areas, so that the atrophy appeared to be the result solely of spontaneous, reciprocal lymphocytic hypoplasia. Conversely, the administration of foreign protein to rabbits[17] resulted in a marked increase in circulating lymphocytes at the expense of granulocytes, the latter falling as low as 400 per c. mm. during the peak of lymphocytosis. Autopsy surveys showed an extensive, marked, generalized hyperplasia of all lymphoid tissues including a greatly enlarged spleen, while the bone marrow everywhere showed a marked myelocytic hypoplasia extending to complete aplasia in some normally active areas.

Simple, uncomplicated, hypertherm- or radiotherm-induced fever in rabbits has been found to influence myelopoiesis favorably while at the same time destroying lymphocytes and inhibiting their regeneration temporarily.[36] A progressive "left shift" in the nuclear index of the circulating granulocytes was correlated with prompt progressive, myeloid hyperplasia and increased mitotic activity in marrow, and a marked immediate postfebrile leukocytosis; conversely the peripheral lymphopenia reflected a progressive cellular destruction necessitating increased clasmatocytic phagocytosis without regenerative replacement wherever lymphocytes were located in the tissues, with a prolonged latent postfebrile period before lymphopoiesis could again become effectively re-established.

In each of the instances cited, one particular cell strain was markedly responsive to a stimulus, which at the same time, either directly or indirectly, influenced adversely another supposedly closely related cell type. It might seem that were the granulocytic cells directly dependent upon the lymphocyte of blood and lymph nodes for their origin and differentiation, any influence subversive to lymphocytic integrity would likewise be reflected in a diminished production and supply of granulocytes to the organism. Or, if an unusually prolific supply of multipotential lymphocytes became available, there would presumably be no obvious reason for a reciprocal decrease in the direct progeny of such a stem cell; rather some corresponding increase in granulocytes might be anticipated. Quite the opposite has been observed in the experimental field.

Is there, then, any counterpart in the cellular disequilibria occur-

ring during the clinical course of human disease, which might permit of interpretations in one direction or the other concerning fundamental cell origins and cell relationships? In infectious mononucleosis a marked lymphocytosis (Fig. 4), both relative and absolute, usually occurs with characteristic qualitative changes in this cell strain, associated with a more or less generalized lymphadenopathy. A reciprocal granulopenia has been observed, resulting not infrequently in a profound decrease to 500 or less granulocytes per c. mm., the sternal bone marrow showing at such times an absolute decrease in myelocytes.[33] Clinical recovery is not complete until the disturbed cell relationships in the blood and tissues have been corrected.

The Mechanism and Specificity of Cell Stimulation and Cell Inhibition

Turning to the problem of acute granulopenia in human disease, the nucleotides may be said to have the strongest and most powerful chemotactic and maturative stimulus for neutrophilic granulocytes of any agent thus far studied. In patients with the Schultz syndrome,[37] if monocytes remain present in the peripheral blood and the sternal bone marrow is not entirely aplastic for myeloid elements, recovery more often than not parallels the administration of pentnucleotides. This statement is made with full realization of the establishment of a specific hypersensitive destructive and inhibitory etiology for amidopyrine and certain other drugs in certain specific myelopenic states, with the desirability and necessity of prevention in these cases. In all leukopenic patients, in which the etiology is not immediately obvious, however, a knowledge of the state of the bone marrow is essential to intelligent therapy today. Usually this information may be obtained quite as satisfactorily by sternal puncture as by surgical trephine. The principal requirement is a familiarity with the morphologic characteristics of the maturation cycle of each strain of cells found normally in bone marrow and with the relative proportions of each as revealed by actual differential cell counts of representative marrow samples.

When the peripheral leukopenia is found to be secondary to both a maturation arrest and an inhibition of marrow myelopoiesis, the recovery, if it occurs, is reflected by changes in the granulocytes, which are entirely comparable to those observed in the red cells during the recovery from relapse in pernicious anemia. Depending upon the degree

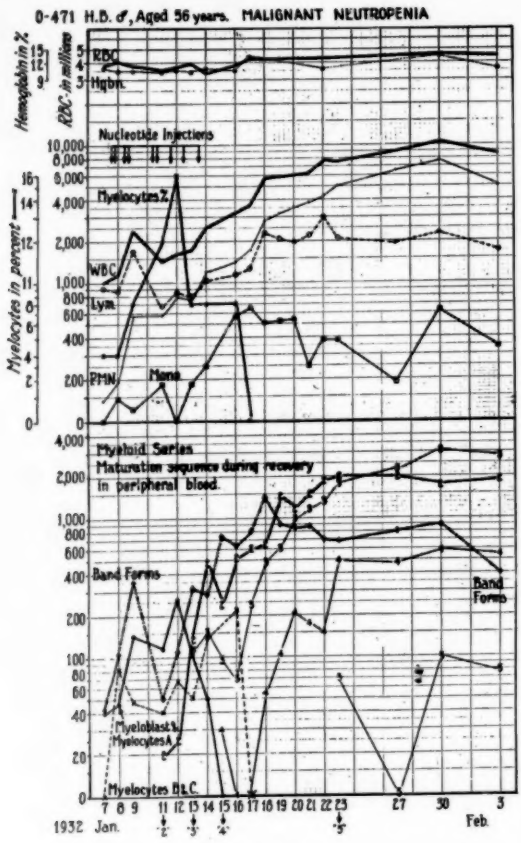

Fig. 7

This, in detail, is the peripheral blood reflection of bone marrow recovery in a classical acute neutropenic episode of undetermined etiology. There appeared sequentially in the blood every stage in the entire life cycle of the neutrophilic granulocyte, from myeloblast to mature, five-lobed polymorphonuclear, the myelocyte percentage rising to a peak and then dropping sharply in duplication of the reticulocyte phenomenon illustrated in Fig. 6. From such observations it is estimated that it may take as long as 16 days for maturation from myeloblast to five-lobed neutrophil, and that regeneration must take place, in some instances at least, from the myeloblast level.

of myelocytic immaturity which prevails at the time of the reactivation of the marrow, there will appear transitorily in the peripheral blood, myelocytes in increasing percentage, comparable to a reticulocyte peak.[38] Moreover, the maturation sequence from myeloblast through myelocytes A, B, and C may be followed both in serial marrow punctures and in the daily white cell differential counts. In one such patient

(Fig. 7), when first seen in an acute granulopenic episode with less than 1000 leukocytes per c. mm. (erythrocytes and thrombocytes normal), myeloblasts and myelocytes A were present in the peripheral blood. During the days following the institution of nucleotide therapy, there appeared a maturation sequence in the circulating myeloid elements, which reflected accurately the steps of recovery in the marrow. The myeloblasts and the earliest myelocytes with the first few specific granules increased to their highest point of 280 per c. mm. on the fifth day of treatment, disappearing on the eighth. Myelocytes B and C, containing a more complete complement of specific granules, were present at the end of the first 24 hours, reached their maximum of 250 on the ninth day, and had disappeared by the following day. The "band forms" of Schilling, the youngest of the motile mature granulocytes, were present to the number of 40 per c. mm. on the first examination and were found in all preparations at all times, but did not show any sustained absolute increase until the eighth day, reached their peak of 1400 on the eleventh day, and thereafter gradually decreased to resume their proportionate representation as equilibrium was again established. The first granulocyte with a segmented nucleus, two lobes, was observed on the fourth day, and thereafter these two-lobed neutrophils increased steadily until the twentieth day, when the three-lobed granulocytes, which had been present since the sixth day, finally surpassed them in total number. One cell with four lobes to the nucleus was seen in one preparation on the eighth day, but not until the eleventh day were they permanently present. Five days later, on the sixteenth day following the beginning of recovery, granulocytes showing five nuclear segments appeared, at which time the total granulocytes had increased from their original low of less than 100 to something over 5000 per c. mm. Thus, from myeloblast to five-lobed polymorphonuclear neutrophilic leukocyte required approximately 2 weeks' time in this individual. With increasing maturity of the circulating myeloid cells came increased absolute numbers, reflecting both a specific myelopoietic inhibition and maturation arrest in the marrow during the fastigium of clinical symptoms. The episode described, occurred in January, 1932 unrelated to any determinable drug etiology, and though two less severe leukopenic relapses occurred during the following two years, associated with severe emotional environmental stresses and strains, this physician-patient is still living and well at the time of this writing, with no evidence of hemo-

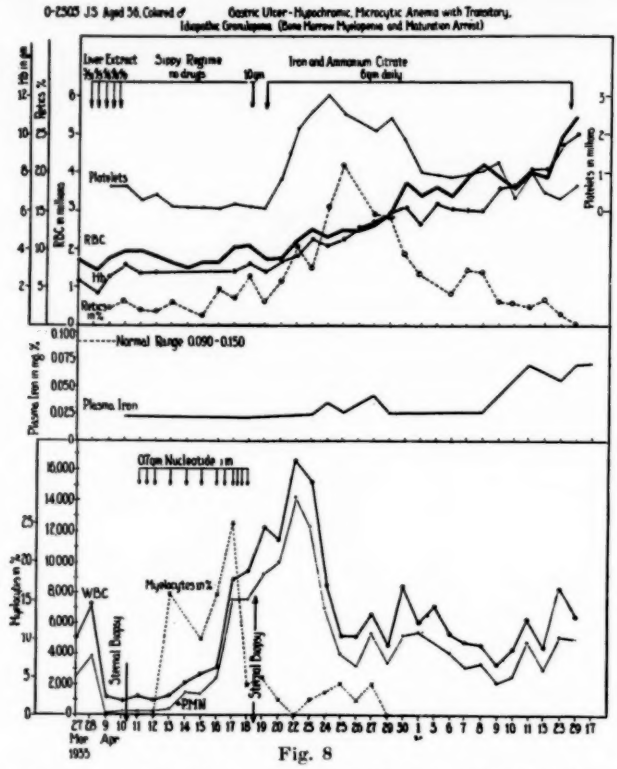

Fig. 8

Demonstration of the complete independence of myelopoietic and erythropoietic responses to specific therapy in an individual developing an acute idiopathic granulopenia while in the hospital under observation for a severe anemia secondary to bleeding peptic ulcer. Liver extract neither helped the anemia nor prevented nor "cured" the neutropenia. The extremely low plasma iron reflects the exhaustion of the body's iron reserves from chronic hemorrhage resulting in a typical, microcytic, hypochromic anemia. Note different time relationships in the administration of the various therapeutic agents and the corresponding cellular responses, including the platelet increase which accompanied the erythroid recovery. See Table I for the actual differential counts of blood and sternal marrow cells April 10 and 18.

poietic marrow inadequacy.

The specificity of the inhibition of marrow myelopoiesis in these clinical instances of granulopenia has been stressed, thus emphasizing two significant points with reference to the physiology of bone marrow as an organ, namely, the probable independence of the respective stem cells from which erythrocytes and granulocytes normally arise throughout postembryonic life, and, an obvious corollary, the specificity of the responses to both depressants and stimuli of these respective cell strains. Fig. 8 and Table I further support these conclusions. During

TABLE I

(To be studied in conjunction with Fig. 8 and the text)

NUCLEOTIDE-TREATED IDIOPATHIC NEUTROPENIA
(maturation arrest)

Serial Sternal Bone Marrow Biopsies

Patient: J. S. Aged 36 years, colored, ♂.

Diagnosis: 1—Gastric ulcer—hypochromic microcytic anemia.
2—Idiopathic granulopenia, acute, transitory (no drugs).

April 10	1935	April 18
BLOOD		
Total WBC 925		9400
Total RBC.....1,700,000		2,090,000
Hemoglobin		2.8 gm./100 cc.
(Newcomer) ..2.5 gm./100 cc.	PMN 81%	Lym. small 8%
Supravital Diff...Only 1 2-lobed PMN found	PMB 1	int. 1
	Myelocytes 3	Monocytes 6

BONE MARROW

Supravital Diff.	First Biopsy			Second Biopsy		
PMN	13	4%		76	14%	
Metamyelocyte	6	1%	15%	68	12%	94%
Neutrophilic "C"	30	9%		360	67%	
"B"	246	71%		32	6%	
"A"	51	14%	85%	0	0%	6%
Basophilic "C"	1			12		
"B"	13			4		
"A"	10			2		
Eosinophilic "C"	1			8		
"B"	1			2		
"A"	0			0		
Myeloblast	1			0		
Lymphocyte	2			0		
Monocyte	1			2		
Clasmatocyte	16			4		
Primitive Cell	0			0		
Total WBC	392		36%	572		54%
Normoblast	595	87%		354	75%	
Erythroblast	62	9%		116	24%	
Megaloblast	23	3%		4	0.8%	
Total RBC	680		64%	474		46%
Cells counted	1072			1046		

8.4 gm. nucleotide given i.m. in 8 day period

hospitalization, incident to a severe hypochromic, microcytic anemia secondary to bleeding peptic ulcer, there developed in this patient an acute granulopenic episode. Sternal biopsy proved the accuracy with which the peripheral blood was reflecting the underlying marrow pathology. Only 36 per cent of all nucleated marrow elements were of the myeloid series and 85 per cent of these were the very young myelocytes A and B, a distinct "left shift" from the normal maturative relationships. The nucleated red cells (64 per cent of all marrow elements) were predominantly (87 per cent) at the normoblastic level, which is representative of the maturation level when no inadequacy in the erythrocyte maturation factor prevails, and explains the lack of response to liver extract, given as a control. Eight days later, when the granulocytes had increased in the peripheral blood from practically zero to 8000 per c. mm., a second sternal biopsy from another interspace revealed a corresponding change in the age and proportionate representation of the myeloid elements without significant qualitative alteration in erythropoiesis or circulating erythrocyte level. The myelocytes represented 54 per cent of all nucleated cells at this time, and of equal or even greater significance was the increase in the relative proportion of the more mature myelocytes C and metamyelocytes from 15 per cent to 94 per cent. During this change in the number and maturity of myelocytes in the marrow, the peripheral blood was showing a myelocyte peak with a high of 25 per cent reached on the seventh day following institution of nucleotide therapy, preceding the absolute increase in total circulating granulocytes, much as does the reticulocyte rise following specific therapy in the anemias. During this episode, erythrocytes and hemoglobin remained stationary. However, with the institution of adequate iron therapy in this patient, a typical reticulocyte response was elicited, reaching a high of 21 per cent on the seventh day followed by a rapid return to normal in plasma iron, total red cells and hemoglobin. No further hematologic disturbances have been observed in this patient.

The occasional chronic lymphatic leukemia patient will have a bone marrow hypoplastic for myelocytes unassociated with lymphocytic hyperplasia or infiltration. In such a case[39] it was possible to stimulate a rise in neutrophilic granulocytes from a few hundred to 10,000 per c. mm. with daily injections of the nucleotides (Fig. 9). No demonstrable effect on the lymphocytes was observed, suggesting the essen-

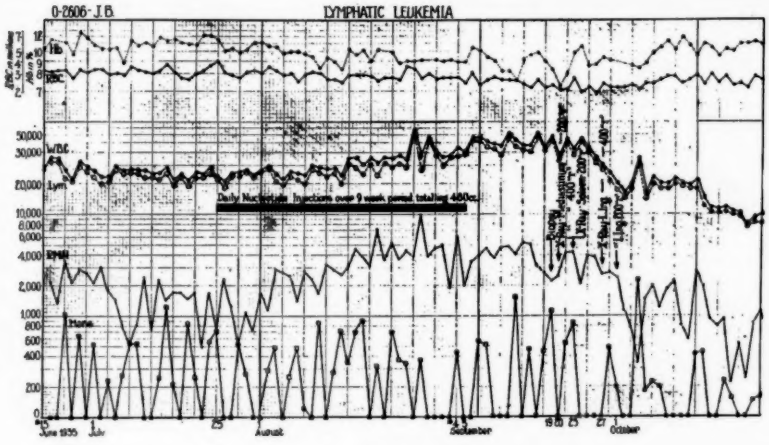

Fig. 9

A patient with chronic lymphatic leukemia with reciprocal granulopenia as low as 500 neutrophils per c.mm., during 9 weeks of daily nucleotide injections showed a gradual but steady increase in these elements to 10,000 per c.mm. During this period there was no reciprocal fall in the leukemic lymphocytes, but rather a slow uninterrupted increase, which continued until deep x-ray therapy was administered. The polymorphonuclear neutrophils failed to continue their upward trend as soon as nucleotide therapy was discontinued, and subsequently fell to their previous low level, indicating the specific character of the stimuli, and independent capacity to respond, of these two cell strains.

tial independence of lymphocytic and myelocytic responses, at least under certain circumstances. The same fundamental observation has been made relative to lymph node atrophy and lymphocytic hypoplasia in chronic myeloid leukemia. In this disease a profound peripheral lymphopenia is the rule, which reflects the reciprocal cell relationships as found in the hemopoietic tissues. Again, in certain patients showing pan-marrow hypoplasia, with anemia, lymphocytic leukopenia, a mild lymphadenopathy, and the presence of a scattering of lymphocytes in the otherwise aplastic bone marrow, this spontaneous "pseudoleukemic" myeloid-lymphoid reciprocity has at times raised the question of specifically stimulated lymphatic hyperplasia.

Reports of an occasional toxic disturbance of normal marrow function during sulfanilamide medication are appearing in the medical literature. One such instance in our own experience serves to emphasize the difference in character and in time relationships of the various marrow element responses when such a reaction occurs. A 66 year old, colored, male patient entered the hospital, on the fifth day following mild trauma, critically ill with a proven beta-hemolytic streptococcus

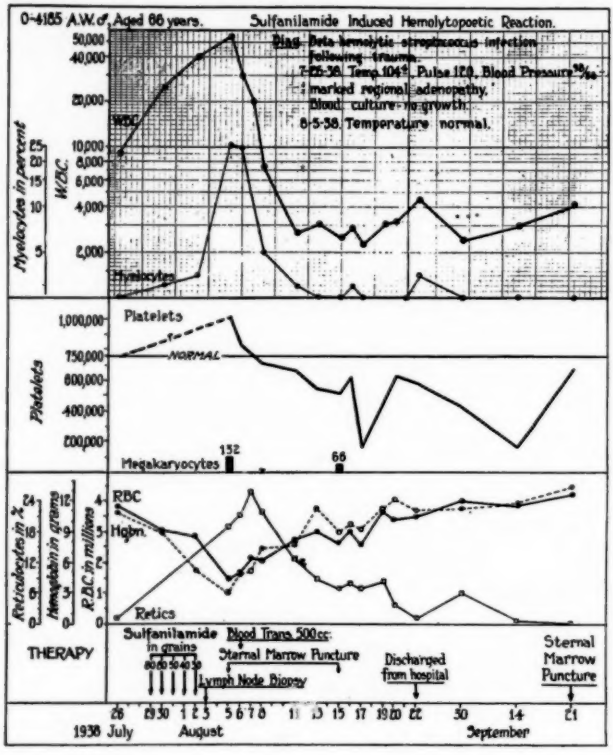

Fig. 10

This patient illustrates the difference in type, degree and time relationships of the reaction of the various bone marrow elements to the same toxic agent. At the point of maximum hemolysis, with red cells at a million and a half, hemoglobin 3 gms., and a spontaneous reticulocyte peak of 24 per cent, the total white cell count was 55,000 with 27 per cent myelocytes, and the platelets were over a million per c.mm. By the time the red cells had again reached a normal equilibrium a persistent leukopenia and thrombocytopenia had developed. See Table 2 for the correlation of peripheral blood and sternal bone marrow data taken at representative periods.

infection involving the affected extremity and the regional lymph nodes. The total white cells were only 9000 per c. mm., granulocytes 85 per cent with 11 per cent band forms and numerous toxic granule cells; red cells and reticulocytes were within normal limits. Sulfanilamide was administered in diminishing dosage as indicated in Fig. 10 for 5 days, during which period clinical improvement was prompt and striking, and coincided with a marked rise in circulating white blood cells to a high point of 55,000, with 65 per cent neutrophilic granulocytes, 25 per cent

TABLE II

(To be studied in relation to Fig. 10 and the text)

SULFANILAMIDE INDUCED HEMOLYTOPOIETIC REACTION

Serial Sternal Bone Marrow Biopsies

Patient: A. W. Aged 66 years, colored, ♂.

Diagnosis: Beta hemolytic streptococcus infection following trauma. Marked regional adenopathy. Blood culture—no growth.

1938	August 5	August 15	September 21
BLOOD			
Total WBC.	55,000	2,500	4,250
Total RBC.	1,570,000	2,660,000	4,250,000
Total Platelets	1,070,000	526,680	680,000
Hemoglobin	3 gm./100 cc.	9 gm./100 cc.	13.2 gm./100 cc.
Reticulocytes	19.8	7.4	0.2
Supravital Diff.			
PMN	65%	60%	58%
PMB	0	—	1
Myelocytes	27	0	0
Monocytes	0	10	8
Lymphocytes	8	30	33

BONE MARROW

Supravital Diff.	First Biopsy	Second Biopsy	Third Biopsy
PMN	73%	48%	46%
Neutrophilic "C"	22.5	18	36
"B"	2	2	0
"A"	0	2	0.5
PMB	0.5	2	0.5
PME	1	6	0.5
Eosinophilic "C"	0	0	0.5
"B"	0	0	0.5
Myeloblast	0	1	0
Lymphocyte	0	14	13.5
Monocyte	0	0	0.5
Clasmatocyte	**1**	**5**	**0.5**
Plasma Cell	0	2	1
WBC Ratio	**27%**	**29%**	**74%**
Normoblast	68%	50%	44%
Erythroblast	32	50	56
Megaloblast	0	0	0
RBC Ratio	**73%**	**71%**	**26%**

280 grains sulfanilamide given orally in 5 days (July 29-Aug. 3).

of which were myelocytes. The platelets were found at this time to be somewhat higher than normal. The red cells meantime had fallen sharply to a million and a half, the hemoglobin to 3 gms. per 100 cc., while the reticulocytes, reflecting an increased spontaneous compensatory marrow delivery of new young erythrocytes, had risen to 19.8 per cent. As the red cells rapidly increased on the rebound, the leukocytes fell to a definite leukopenic level, which was maintained for some 6 weeks thereafter. The platelets paralleled the falling white cells and remained below normal for a comparable period. On the first two sternal marrow studies, the myeloid cells represented less than 30 per cent of all nucleated cells encountered, while by September 21 the proportion of erythroid to myeloid elements had again returned to normal. Thus, there were stimulatory, inhibitory and destructive phases in the reactions, differing in degree and duration with the cell types involved. In this instance one may hypothesize the sulfanilamide as increasing the effectiveness of, and the need for phagocytic leukocytes, at the same time diminishing the toxic myeloid inhibiting effects of the streptococci; conversely, the drug proved hemolytic for red cells in this individual in the dosage given, with the development of an acute, transitory anemia. Following the subsidence of the infection and the elimination of the therapeutic drug, the marrow returned more or less promptly to its original equilibrium.

Much more information needs to be accumulated relative to the white cells before we shall be in a position to influence their life cycles at the source as beneficially as is now possible for the red blood cells. At the moment, the important fact is their apparent independent response to various physiologic and pathologic influences, and their separate, even though closely related, functional specificities and capacities.

The Hemolytopoietic Equilibrium

It must be clear to anyone familiar with the so-called hemolytopoietic equilibrium, that the problem is only a little more than half solved when the cell production end of the mechanism alone has been considered. Optimum peripheral distribution and minimal destruction of the cells once delivered to the circulation are fully as important for the maintenance of health. The spleen acts physiologically as the chief sequestration and reservoir organ for the blood cells, and its normal content of phagocytic clasmatocytes is greater proportionately, and in the aggregate, than any other organ in the human body. This furnishes the back-

ground for the well known splenic function of senile cell disposition, but at the same time sets the stage for a series of disease entities dependent upon the pathologic overdevelopment of one or more of these normal "graveyard" functions. In congenital hemolytic icterus, if the spectacular clinical recovery which follows successful splenectomy,[39] is correctly interpreted, it is this surgical removal of an organ with an inherited tendency to excessive hemolysis, rather than any direct attack on bone marrow deficiency or inadequacy, which restores the cellular equilibrium. The bone marrow is hyperplastic for red cells at the late erythroblast and normoblast levels of maturation during active anemic episodes with jaundice, and in the absence of all splenic tissue, the marrow has been proved to be entirely competent to maintain a normal cellular balance, adequate for health and for resisting successfully infection, despite red cell size or fragility abnormalities.

In certain instances of thrombocytopenic purpura where the marrow can be shown to contain the usual, or excessive numbers of qualitatively normal megakaryocytes, the successful removal of the spleen restores promptly and permanently, an adequate supply of functionally normal platelets to the circulation.[40]

Finally, in selected patients showing a profound granulopenia of otherwise undetermined etiology, with or without hepatic cirrhosis, but practically always with a marked splenomegaly, a more or less specific phagocytosis of granulocytes by excessive numbers of clasmatocytes can be demonstrated, both histologically in the excised splenic tissue, and clinically through the subsequent restoration of a normal peripheral white cell count and differential, the bone marrow having been found to be hyperplastic for myeloid cells in each patient throughout the preoperative leukopenic episode.[41]

Thus, when any deficit occurs in the essential blood elements in the peripheral circulation, and bone marrow studies reveal an hyperplasia of qualitatively normal precursors, the organ of origin is automatically cleared in the great majority of instances, and some peripheral etiologic factor or factors must be sought. The more extensive our knowledge of the specificity of functions and sensitivity of response of the essential cells of blood and connective tissues, the more effective should become the scientific control of the great variety of noxious states most of which influence profoundly and are thereby in turn significantly affected by the reactions of the cells we have been considering. The problems of

functional differentiation and morphologic identification are no longer of academic interest only. The accumulated information now available, at its best is life saving and specifically curative when intelligently and discriminatingly applied; at second best, it places clinical prognosis on a sounder scientific basis and points the way to the next steps wherein may lie the more complete answers to the many questions and problems still challenging solution.

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THE CURIOUS CAREER OF TYPHOID MARY*

GEORGE A. SOPER

IN spite of the fact that Mary Mallon was the most famous typhoid carrier who ever lived, the world knows very little about her. There is the paper in which I announced her discovery, published in the *Journal of the American Medical Association*, June 15, 1907, and the article in which I traced her career up to her final incarceration by the City of New York, eight years later, published in *The Military Surgeon* for July, 1919; but in the innumerable printed references which have appeared in the last thirty years these authoritative sources have generally not been fully utilized. Errors have been committed where one would least expect them and, these being copied and sometimes elaborated on, there have been woven accounts which depart materially from the facts. In my way of thinking the truth about Typhoid Mary is far more interesting than the tales which have been imagined about her, and as all the essential facts have not yet been told, I am glad to tell you some of the things which I think medical men may be interested in.

I first saw Mary Mallon thirty-two years ago, that is, in 1907. She was then about forty years of age and at the height of her physical and mental faculties. She was five feet six inches tall, a blond with clear blue eyes, a healthy color and a somewhat determined mouth and jaw. Mary had a good figure and might have been called athletic had she not been a little too heavy. She prided herself on her strength and endurance, and at that time and for many years thereafter never spared herself in the exercise of it. Nothing was so distinctive about her as her walk, unless it was her mind. The two had a peculiarity in common. Those who knew her best in the long years of her custody said Mary walked more like a man than a woman and that her mind had a distinctly masculine character, also.

I think Mary was born in the north of Ireland. She could write an excellent letter, so far as composition and spelling were concerned. She wrote in a large, clear, bold hand, and with remarkable uniformity. She

* Read May 10, 1939 before the Section of Historical and Cultural Medicine.

read a good deal in the days of her captivity and seldom missed her daily paper.

At the time I knew her, Mary had no home. If she had relatives or friends in this country or Europe, she never revealed the fact. Twice I thought I talked with a sister; once in Bridgeport, Connecticut, where my investigations carried me, and once in Brooklyn; but I could not prove it. At the time of her death relatives were advertised for in two newspapers for one month, by order of the Surrogate of Bronx County, and a lawyer was appointed to protect their interests, for Mary left a little money, but none appeared. None ever came forward when she was sick or in other trouble, and she never sent for any.

My discovery of Typhoid Mary was the outcome of an investigation made in the winter of 1906-'07 into an outbreak of typhoid fever in the house of Mrs. George Thompson, at Oyster Bay, N. Y., the preceding summer. The place had been rented to a New York banker, General William Henry Warren, who had occupied it with his family of three, and seven servants for the summer months. Late in August an explosion of typhoid had occurred in which six of the eleven persons in the household were taken sick. The epidemic had been studied immediately after it occurred by persons who were regarded as experts, and there were a number of typewritten reports upon it, but the cause had not been positively ascertained. It was thought by the owner that unless the mystery could be cleared up, it would be impossible to find tenants for the coming season.

It will be remembered that in those days typhoid fever was far more common than it is today and that the knowledge of its transmission was less complete. The reported cases in New York City in 1906 were 3,467 and the reported deaths, 639. The actual amount of typhoid was probably much greater.

Typhoid was believed to be due generally to polluted water or milk, or, in the opinion of some, to putrefying organic matter and sometimes to sewer gas. A paper was presented before the Royal Society by Horrocks at the end of 1906, in which he reported that typhoid and other bacteria might be carried by sewer air after being disengaged from the sewage or walls of the sewers, and the *Journal of the American Medical Association* declared that this possibility should be remembered.

At the end of the century typhoid epidemics, occasionally of hundreds of cases, were occurring in different parts of the country. They

were frequent among students of colleges and universities, and now that it is all over it is amusing to see the attempts that were made by college and town authorities to shift the responsibility for them.

Also there were villages and isolated houses to which typhoid clung with inexplicable pertinacity, and solitary cases or small groups appearing here and there in healthy communities for which no explanation could be found.

It was to be Mary Mallon's fate to clear away much of the mystery which surrounded the transmission of typhoid fever and to call attention to the fact that it was often persons rather than things who offered the proper explanation when the disease occurred in endemic, sporadic and epidemic form.

It happened that in 1907 I had had a good deal of experience with typhoid fever. This began when as an undergraduate student passing my Christmas holidays at Warrensburg in the Adirondacks, I had the temerity to move two typhoid patients and their families out of a house which had a long history of communicable disease and, with the consent of the owner, burn it to the ground. My experience grew with my years. Eventually I was called on to investigate and put a stop to many epidemics, numbering the City of New York and the State of New York among my clients. I was called an epidemic fighter.

When I went to Oyster Bay, the first thing I did was to get together all the essential facts about the outbreak: the dates of attack, the diagnoses, and like information. The next was to check over the possible sources and channels of infection as suspected and studied by preceding investigators. This check up was done with the utmost care, my assumption being that somebody might have overlooked some important detail.

I was disappointed. They had done their work thoroughly. Try as I would, I could not find anything wrong. The nearest explanation was that an old Indian woman who lived on the beach had brought polluted shellfish to the household, but this theory would not stand the rigorous inquiry which I gave it.

So I turned my attention from the well, the overhead tank, the cesspool, the privy, the manure on the lawn, the food supplies, the bathing and the sanitary condition of the neighboring property to the people in the house. I thought it probable that some event outside of the usual routine in this well-regulated household had occurred a little before the outbreak began. Had a carrier come to the house?

The idea of carriers was not entirely new to me. I had taken care to guard against carriers – urinary carriers – in my epidemic work. As is well known, a considerable percentage of convalescents pass a highly infected urine for many weeks after they are sufficiently recovered to go about; so I had had urotropin put up in convenient form to administer and issued an order that no patient might be discharged from medical attention until his urine was proved to be bacillus-free.

It was hard to identify typhoid in the feces by current bacteriological methods. I had read an address which Koch had delivered in 1902 on typhoid investigations in Trier which were based on a paper by Conradi and Drigalski in the same year, setting forth results they had obtained with a new culture medium in examining the stools of apparently well persons. A *Festschrift* on the sixtieth birthday of Koch, which appeared in 1903, contains several papers on the probable role of healthy carriers in producing typhoid, and publications by others in Germany were to like effect. Most of these I had not seen until Dr. Simon Flexner called my attention to them after I had concluded my work on the Mary Mallon case.

Nothing of the kind had been done in America and the discovery of Typhoid Mary brought to light for the first time in America, or any other English-speaking country, a chronic typhoid carrier with infected feces. This type of carrier is now regarded as by all means the most common.

Having undertaken to see if there had been any carriers in the Oyster Bay house before the outbreak there occurred, I soon came, through the process of exclusion, to the cook. But where was she? She had left soon after the epidemic and that event had occurred over six months ago. I tried to find out everything I could about her, but there was not much to learn. Mrs. Warren said she was a good plain cook, her wages were forty-five dollars a month, and she had been obtained from Mrs. Stricker's. Stricker's was a well-known employment agency on Twenty-eighth Street. The cook had not fraternized with the other servants and they knew little about her. She was not particularly clean. Her name was Mary Mallon. That was about all.

But some of the details were of great significance. The cook had come to work on August 4, the first person fell ill August 27, and the last September 3. It seemed probable that all of the patients, the dates of whose attacks fell within a period of seven days, were infected at the

same time and that this was after the new cook's arrival.

It was not at first clear how the family could have been infected from the cook, granting that she was a carrier, for, where there are so many servants, there is little food that a cook handles which is not subsequently raised to a temperature sufficient to make it harmless. I found, however, that on a certain Sunday there was a dessert which Mary prepared and of which everybody present was extremely fond. This was ice-cream with fresh peaches cut up and frozen in it. I suppose no better way could be found for a cook to cleanse her hands of microbes and infect a family.

I went to the employment agency which had sent the cook and explained the situation. I asked for Mary's references, for I wanted to know where else she had worked. I obtained all the help that could be given there, but it was not very much.

Mary appeared to be a person who moved about a good deal; she did not remain long in any situation. She did not get all her situations through one agency, or any agency for that matter. Sometimes they came through advertisements.

I found some of the places where Mary Mallon had worked and asked if anything unusual had happened there. I would like to describe at length what I learned, but it would take too long. I uncovered a series of seven household epidemics. They were alike in the unexpectedness with which the cases occurred, the complete ignorance as to the source, the apprehension as to who would be next, the suffering of the patients, the disruption of the household arrangements, the general bewilderment. The cook had, of course, never been suspected as the cause of any of these epidemics.

My earliest record places Mary Mallon at Mamaroneck, where a New York family had a house for the summer. A young man made a visit there and came down with typhoid ten days after his arrival. The date was September 4, 1900. At the time it was thought that he had contracted his disease during a visit to East Hampton, because that place was at no great distance from the Montauk army camp where typhoid was prevalent. No definite route of infection was indicated.

In 1901-'02, Mary lived about eleven months with a family in New York City. A laundress was taken to Roosevelt Hospital with typhoid, December 9, 1901. The case was seen by Dr. R. J. Carlisle, but not investigated.

In 1902 Mary was taken to Dark Harbor, Maine, to a new house that had been rented for the summer by Coleman Drayton, a lawyer of New York City. A case of typhoid fever occurred June 17, two weeks after the cook's arrival. Seven days later a second case occurred. Two days after that there was a third. Soon seven out of the household of nine were sick, the only two escaping being Mr. Drayton, who had had typhoid some years before and was presumably immune, and the cook. A trained nurse followed and a woman who came to work by the day was attacked. The outbreak was investigated at the time by Dr. E. A. Daniels of Boston and Dr. Louis Starr of Philadelphia. The first case was that of a footman; some believed that he had become infected outside and brought the sickness to the house. Dr. Daniels thought the first three cases were simultaneously infected somewhere; but on checking over his report I could not agree with him. I found that the three had not eaten the same food or drunk the same water.

As may be imagined, the household was pretty well upset. Proper help could not be had. Mr. Drayton and Mary Mallon worked side by side taking care of the sick and attending to all the manifold tasks which illness brings upon a family. Mr. Drayton told me that when it was over he had been so grateful to Mary for all the help she had given him that he rewarded her with fifty dollars in addition to her full wages.

In 1904 Mary figured in an outbreak in the household of Mr. Henry Gilsey at Sands Point, Long Island. There were, all told, eleven persons on the place: four in the family and seven servants. The cook arrived on June 1 and on June 8 the laundress, who had been on the place ten days, fell ill of typhoid. Then followed the gardener, the butler's wife, and finally the butler's wife's sister. There were four, all told, who fell ill within three weeks in this outbreak. The cases were all among the servants, and since they lived in a house apart from the family, it was thought that there must be something wrong about their house. Several persons investigated this outbreak, including Dr. R. L. Wilson, superintendent of hospitals for communicable diseases of the New York City Department of Health. His opinion was that the laundress must have become infected before she entered this employment, but, although he tried, he could not find how this could have happened.

After leaving Oyster Bay, Mary Mallon was employed as a cook at Tuxedo. She remained from September 21 to October 27, 1906. Fourteen days after her arrival a laundress was removed with typhoid to

St. Joseph's Hospital at Patterson. She was seen by Dr. E. C. Rushmore.

Here was a curious group of epidemics. In nearly every instance, a well-to-do and socially prominent family, soon after moving from the city to the country for the summer, experienced an outbreak of typhoid fever. In no instance had its cause been satisfactorily explained. The cook always left soon afterward. She had never been suspected.

When at length I caught up with her, which was some four months after I started out on the Oyster Bay epidemic, Mary was working as cook in an old-fashioned, high-stoop house on Park Avenue on the west side, two doors above the church at Sixtieth Street. The laundress had recently been taken to the Presbyterian Hospital with typhoid fever and the only child of the family, a lovely daughter, was dying of it.

I had my first talk with Mary in the kitchen of this house. I suppose it was an unusual kind of interview, particularly when the place is taken into consideration. I was as diplomatic as possible, but I had to say I suspected her of making people sick and that I wanted specimens of her urine, feces and blood.

It did not take Mary long to react to this suggestion. She seized a carving fork and advanced in my direction. I passed rapidly down the long narrow hall, through the tall iron gate, out through the area and so to the sidewalk. I felt rather lucky to escape.

I confessed to myself that I had made a bad start. Apparently Mary did not understand that I wanted to help her. It mattered not that I told her if she would answer my questions and give me the specimens, I would see that she got good medical attention, in case that was called for, and without any cost to her.

As a matter of fact, I did not need the specimens in order to prove that Mary was a focus of typhoid germs. My epidemiological evidence had proved that. Laboriously I had worked out every one of the seven outbreaks and I was positive that Mary had produced them all. Just exactly how she did it I didn't know. I wanted to find out. No doubt her hands played a part in it. They became soiled when she visited the toilet, but whether from her urine or feces I had no way of knowing.

I felt a good deal of responsibility about the case. Under suitable conditions Mary might precipitate a great epidemic.

I found that Mary was in the habit of going, when her work for the day was finished, to a rooming house on Third Avenue below Thirty-third Street, where she was spending the evenings with a disreputable

looking man who had a room on the top floor and to whom she was taking food. His headquarters during the day was in a saloon on the corner. I got to be well acquainted with him. He took me to see the room. I should not care to see another like it. It was a place of dirt and disorder. It was not improved by the presence of a large dog of which Mary was said to be very fond.

I made an arrangement with Mary's friend to meet her in this room; and taking an old assistant, Dr. B. Raymond Hoobler, later head of the Children's Hospital at Detroit, I waited one evening for Mary at the top of the stairs.

Mary was angry at the unexpected sight of me, and although I recited some well considered speeches committed to memory in advance to make sure she understood what I meant, and that I meant her no harm, I could do nothing with her.

She denied she knew anything about typhoid. She had never had it nor produced it. There had been no more typhoid where she was than anywhere else. There was typhoid fever everywhere. Nobody had ever accused her of causing any cases or had any occasion to do so. Such a thing had never been heard of. She was in perfect health and there was no sign or symptom of any disease about her. And she would not allow anybody to accuse her. Again I saw I was making no headway, so Dr. Hoobler and I left, followed by a volley of imprecations from the head of the stairs.

Learning that Mary was going to leave her Park Avenue situation and knowing that if she did so it would be hard to find her again, I brought her case to the attention of Commissioner Thomas Darlington and Dr. Hermann M. Biggs, Medical Officer of the New York City Health Department, and recommended that Mary Mallon be taken into custody. I wanted to have her excretions examined by Dr. William H. Park at the Department's Research Laboratory. I called Mary a living culture tube and chronic typhoid germ producer. I said she was a proved menace to the community. It was impossible to deal with her in a reasonable and peaceful way, and if the Department meant to examine her, it must be prepared to use force and plenty of it.

The Department acted favorably on my recommendation. It would get the specimens peacefully, if possible, but if this was not possible it would get them anyway.

An inspector in the person of Dr. S. Josephine Baker was sent to see

Mary about it. But the success of this gentle yet redoubtable warrior was at first no greater than mine had been. Mary slammed the door in her face.

Next morning a Department of Health ambulance drew up quietly in Sixtieth Street beside the church, with three policemen under Dr. Baker's orders. Two of the policemen were carefully placed so as to prevent Mary from escaping, and one was taken by Dr. Baker with her to the front door. They rang the basement bell and Mary opened the door before she saw who was there. Then she attempted to shut it but the policeman interposed his foot. Mary ran back to the kitchen and disappeared. The doctor and policeman followed quickly, but could not find her. Other servants were there, but they declared they had not seen her. A search was made of the cellar, coal bins and closets, but without result.

On looking out of a rear window Dr. Baker noticed a chair that had been drawn up beside the high fence which separated the property from that adjoining. The ground was covered with snow, and footprints in it led from the house to the chair. Dr. Baker went into the next house and made a thorough search of it but Mary was not to be found. By this time some three hours had been spent in hunting for her.

Dr. Baker then went out into the street and found another policeman and the search was renewed. The hunt was about to be given up when a bit of gingham was seen caught in the door of an outside closet in the rear of the second house. Ashcans were piled against the door. When these were removed, there was Mary. To use Dr. Baker's words:

"She fought and struggled and cursed. I tried to explain to her that I only wanted the specimens and that then she could go back home. She again refused and I told the policemen to pick her up and put her in the ambulance. This we did and the ride down to the hospital was quite a wild one."

A few days later I received a telephone call from Dr. Park, which told me that Mary's bowel movements contained a pure culture of the *Bacillus typhosus*. The examinations were made three times a week from the date of her capture, March 20, to November 16, 1907, and only in a few instances were the typhoid organisms not found.

Some weeks after Mary's arrival at the Willard Parker Hospital, where she had been taken on her arrest, I called on her there. She had been placed in one of the outside isolation wards. In view of her actions

when arrested, she was regarded as a dangerous and unreliable person who might try to escape if given a chance. So she was locked up. It was not an attractive or particularly comfortable room and there was no reason why a strong, active woman of forty who felt herself to be in perfect health, should be contented with it. And Mary Mallon was not.

The room, with its white walls and ceiling and floor, the white bed and the white bathrobe which Mary was wearing, gave the curiously healthy and fearfully angry-looking person a startling appearance.

"Mary," I said, "I've come to talk with you and see if between us we cannot get you out of here. When I have asked you to help me before, you have refused and when others have asked you, you have refused them also. You would not be where you are now if you had not been so obstinate. So throw off your wrong-headed idea and be reasonable. Nobody wants to harm you. You say you have never caused a case of typhoid, but I know you have done so. Nobody thinks you have done it purposely. But you have done it just the same. Many people have been made sick and have suffered a great deal; some have died. You refused to give specimens which would help to clear up the trouble. So you were arrested and brought here and the specimens taken in spite of your resistance. They proved what I charged. Now you must surely see how mistaken you were. Don't you acknowledge it?"

Mary looked at me steadily, but neither spoke nor moved. Her eyes gleamed angrily.

"Well," I continued, "I will tell you how you do it. When you go to the toilet, the germs which grow within your body get upon your fingers, and when you handle food in cooking they get on the food. People who eat this food swallow the germs and get sick. If you would wash your hands after leaving the toilet and before cooking, there might be no trouble. You don't keep your hands clean enough."

Mary's expression did not change nor did she utter a word. I was bound to tell her all that I had come to say, so I continued.

"The germs are probably growing in your gallbladder. The best way to get rid of them is to get rid of the gallbladder. You don't need a gallbladder any more than you need an appendix. There are many people living without them."

"Mary," I continued, "I don't know how long the Department of Health intends to keep you here. I believe that depends partly on you.

I can help you. If you will answer my questions, I will do everything I possibly can to get you out. I will do more than you think. I will write a book about your case. I will not mention your real name; I will carefully hide your identity. I will guarantee that you will get all the profits. It will be easy for you to answer my questions. You know what I want to find out. Above all, I want to know if and when you have had typhoid fever, and how many outbreaks and cases you have seen."

As I finished with my back there against the door, Mary rose. She pulled her bathrobe about her and, not taking her eyes off of mine, slowly opened the door of her toilet and vanished within. The door slammed.

There was no need of my waiting. It was apparent that Mary did not intend to speak to me. So I left the place.

It was not long after my third meeting with Mary that she was sent to Riverside Hospital on North Brother Island. There she was given a little bungalow which had been built for the superintendent of nurses. It consisted of a living room, kitchen and bathroom. It was supplied with gas, modern plumbing and electricity. It was pleasantly situated on the river bank, next to the church.

Mary's food was brought to her; she cooked and ate it alone.

About two years after her arrest Mary sued the City for her release under habeas corpus proceedings, a clever lawyer named George Francis O'Neill bringing suit in the Supreme Court. A strong argument was made on Mary's behalf. It was claimed that she had been imprisoned without due process of law. She had, in fact, not been accused of any crime, nor been given a hearing, nor been represented by counsel.

Mary took the stand and testified that she had never had typhoid fever or caused it in others. She was described by the newspaper reports as robust, bright eyed and energetic.

Dr. Park testified that in spite of appearances and her testimony, Mary was a menace to the community; he had found typhoid bacilli in her excrement and Dr. Soper had traced many typhoid epidemics to her. The judge eventually dismissed the case, saying the Court was unwilling to take the responsibility of releasing her.

When Mary had been incarcerated two years and eleven months, the Health Department released her on her pledge to give up her vocation of cook, not handle the food of others, observe various other precautions and report to the Department every three months. It was

believed that she had had an opportunity by this time to learn all about her condition and how to avoid infecting people.

Various attempts had been made to cure her, but nothing had proved effective. The only thing that remained was to remove the gallbladder, where the germ focus was believed to be situated; but, as Mary would not give her consent to this, it was thought nothing more could be done about the matter.

On her release Mary promptly disappeared. She violated every detail of the pledge she had given to the Department of Health. She changed her name and went to cooking again. Under the name of Marie Breshof, and sometimes Mrs. Brown, she now cooked in hotels, restaurants and sanatoria. At one time she ran a cheap rooming house, but kept it so badly that it failed to pay. She tried ironing, but found cooking paid better.

For five years Mary traveled about New York and its vicinity without restraint and without her identity being discovered by the authorities. I was not asked to find her again, but I think I could have done so. My official connection with the case ended when I cleared up the Oyster Bay outbreak in 1907 and turned Typhoid Mary over to the Health Department. Mary's history during these five years has never been traced in detail, but I know some of the places where she worked and some of the things that happened to her.

The world was not very kind to Mary. She could not resume her cooking in rich private families, for practically all of them got their cooks through two agencies – Mrs. Stricker's and Mrs. Seeley's – and both knew Mary and were afraid to place her.

She never had what might be called a permanent situation. She did not get on well with other servants and wanted to be moving about, anyway.

One day her best friend, a man whose name she often went by, sent for her. He was ill with a bad heart. Mary got him into a hospital, where he died.

She injured her hand; it became infected, and she could not cook for several months. This was too bad, for she had no home and she had little money.

She worked in a Broadway restaurant, a hotel at Southampton, an inn at Huntington, a fashionable hotel in New Jersey, and a sanatorium in New Jersey. There were cases of typhoid which she produced, but

there is no record of all of them. The list includes two children. There was a man for whom Mary mixed a home-made remedy for indigestion and who soon had to be taken off to the hospital with typhoid fever. It is probable that she produced a good many cases in her five-year period of liberty.

One day Dr. Edward B. Cragin, head obstetrician and gynecologist at the Sloane Hospital for Women, telephoned me asking that I come at once to the hospital to see him about a matter of great importance. When I arrived there he said he had a typhoid epidemic of more than twenty cases on his hands. The other servants had jokingly nicknamed the cook "Typhoid Mary." She was out at the moment, but would I recognize her handwriting if she was really that woman? He handed me a letter from which I saw at once that the cook was indeed Mary Mallon, and I also identified her from his description.

I advised that the Health Department be notified, and it was not long before Mary was again taken and sent to North Brother Island. On this occasion she made no struggle.

Mary was on the island the second time for twenty-three years. During this long period she never once tried to escape. Did she want to regain her liberty after her second arrest? I believe she did not.

Some think she had come to recognize her condition as inevitable and had become reconciled to a life of imprisonment.

My belief is that a change had come over her – a change that was due largely to the passage of time. It was both mental and physical. She felt that she had been hounded because of typhoid fever. She did not admit that there was any typhoid about her, but since others said there was, she had not been allowed to go freely where she pleased and do what she wanted to do. As her lawyer had said, she had been advertised to the world as a dangerous person and had been treated worse than a criminal, and yet she had not been guilty of the least violence toward anybody.

Mary was now about forty-eight years of age and a good deal heavier than she was when she slipped through a kitchen full of servants, jumped the back fence and put up a fight with strong young policemen. She was as strong as ever, but she had lost something of that remarkable energy and activity which had characterized her young days and urged her forward to meet undaunted whatever situation the world presented to her. In these eight years since she was first arrested, she had learned

what it was to yield to other wills than her own and to know pain. In the last five years, although she had been free, there had been times when she had found it hard to fight her battles unaided.

On North Brother Island the City afforded her a comfortable place to live – a place where she could cook and sleep and read to her heart's content. Her old age was provided for. There was a good hospital with doctors nearby. She knew by experience that the people on the island would be kind to her. She knew she could keep them from talking about typhoid fever or asking questions about herself.

Mary possessed a violent temper against which, when fully aroused, few persons had ever been willing to contend. I had had this weapon used against me three times, Dr. Baker had seen it in full force on the occasion of Mary's first arrest, and there is a story of it when an English health officer, neglecting a warning he had received, undertook to interview Mary and photograph her at her bungalow. Mary knew how to throw herself into a state of what Dr. John A. Cahill, Superintendent of Riverside Hospital, called, "almost pathological anger."

In the many years of her incarceration, Mary made good use of this personal weapon. Usually a look or a word gave sufficient warning of what might lie behind. When, on the basis of a long and friendly relation, the head of the laboratory asked Mary to tell her about her love affairs, Mary silenced her with a glare.

When Mary returned to North Brother Island she had reason to think that if she showed she would not run away, she might get permission to visit the mainland, and could then see her old haunts in the East Thirty-third Street region, the shops and the streets and the other sights of the city. She could mingle with the crowd as though she belonged in it–as she felt she did.

Whether all this was planned by Mary I do not know, but the fact is she went back to her bungalow and made no fuss about it. She became a privileged guest of the City. Nobody ever talked to her about anything she did not want to talk about. She announced that her past life was a "closed incident," and nobody bothered her about it.

Mary was given a job in the laboratory and learned to make routine simple tests such as all hospitals require. She was paid well for what she did. When she wanted to, she could go to the mainland unattended, and when she came back, nobody made her give an account of herself. Sometimes she would go all the way over to Queens and make a long

visit to a family she knew there. They were not particularly glad to see her.

On Christmas morning, 1932, a man who came to deliver something to her found Typhoid Mary on the floor of her bungalow, paralyzed. She had had a stroke of apoplexy and never walked again. Thereafter, for six years, she was taken care of in the hospital.

She died November 11, 1938. Her body was hurried away and buried in a grave bought for the purpose at St. Raymond's Cemetery in the Bronx. There was no autopsy.

The official death certificate states that the diagnosis was terminal bronchopneumonia of seven days' duration, following chronic nephritis and chronic myocarditis of ten years' standing, and that the fact that the deceased had been a typhoid carrier for twenty-four years had been contributory.

The funeral possessed elements of irony as well as pathos. It was an odd climax to the curious career of this remarkable woman. It was held in the large Roman Catholic Church of St. Luke's, in 138th Street, the Bronx. Only nine persons were present. Of all those in the City employ who had known Mary Mallon and had seen her come and go for so many years, there was not one who followed her to her grave.

The total number of typhoid cases traced to Mary Mallon was fifty-three and the deaths, three. This is not a large toll, compared to the records of some carriers who have since been found. There were doubtless many cases produced by Mary that never came to light.

I could obtain only fragments of her history at the time of my connection with the case, and nobody else seems to have done much to find any more. But if there had been only the Oyster Bay outbreak that was proved to have been caused by her, it would have been enough to make her a woman of mark.

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